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EFFECTS OF CHRONIC ANTIDEPRESSANT DRUG ADMINISTRATION ON GABAERGIC MECHANISMS IN RAT BRAIN

by

DAVID JOHN McMANUS ©

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY
IN

MEDICAL SCIENCES (PSYCHIATRY)

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ABSTRACT

Combined behavioural and neurochemical studies were conducted to investigate the effects of chronically administered monoamine oxidase (MAO) inhibitor [phenelzine (PLZ), tranylcypromine (TCP)] and tricyclic [imipramine (IMI), desmethylimipramine (DMI)] antidepressant drugs on GABAergic mechanisms in rat brain. For comparative purposes, antidepressant drug-induced changes in β-adrenoceptors were also analyzed. *In vivo* tests of β-adrenoceptor and $GABA_B$ receptor function revealed that each antidepressant drug tested induced a decrease in the behavioural effects of β-adrenoceptor stimulation, but did not alter the behavioural effects of $GABA_B$ receptor agonists. Radioligand binding studies carried out with cortical tissue also revealed a differential effect of chronic antidepressant treatment on these two receptor systems. The maximal binding (Bmax) of [^{3}H]-dihydroalprenolol (DHA) to β-adrenergic binding sites was significantly reduced in all antidepressant-treated groups compared to vehicle controls. No significant changes in affinity (Kd) were observed. By contrast, [^{3}H]-GABA binding to $GABA_B$ binding sites was not significantly altered by chronic antidepressant treatment, nor was there an effect on Kd. These data confirm and extend previous reports of reduced β-adrenoceptor number and function following chronic antidepressant treatment, but do not support the proposal that an increase in the total number of $GABA_B$ receptors is a common effect of chronic antidepressant treatment. Analysis of the effects of chronically administered antidepressants on measures of GABA metabolism showed that repeated treatment with PLZ, but not with IMI, DMI nor TCP, induced a decrease in the activity of the GABA catabolic enzyme GABA-transaminase (GABA-T) and significantly elevated GABA levels in cortical tissue. These effects of PLZ on GABA may play a role in its clinical efficacy as an antidepressant/antipanic agent.

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INTRODUCTION

A. GENERAL INTRODUCTION

Depressive illness represents a major subset of psychiatric disorders. A significant proportion of depressed patients responds favorably to antidepressant drug treatments. After initiation of antidepressant treatment there is typically a 2-3 week delay before signs of clinical improvement are observed (Lapierre, 1985). This response delay occurs despite immediate neurochemical effects of the drugs such as inhibition of amine uptake or of enzyme activity (Blier and DeMontigny, 1985). In the last 20 years, progressive changes in monoamine receptors, both pre- and post-synaptic, have been recognized as important biochemical effects of antidepressant drug action that appear to parallel the time-course of clinical improvement (Vetulani *et al.*, 1976; Banerjee *et al.*, 1977; Bergstrom and Kellar, 1979; Charney *et al.*, 1981).

Although chronic antidepressant drug treatments result in a complex spectrum of monoamine receptor changes, it has recently been proposed that a common effect of antidepressants of all classes is an increase in the number of γ-aminobutyric acid (GABA)$_B$ receptors in brain (Lloyd *et al.*, 1985). GABA is an amino acid which acts as a major inhibitory neurotransmitter in the brain (Roberts *et al.*, 1976). As this purported increase in GABA$_B$ receptor number may represent a common feature of chronic antidepressant drug action, it is important to extend and replicate this basic observation and to assess its functional significance. In addition, a growing body of evidence suggests an alteration in GABA metabolism may be operative in both the etiology and pharmacotherapy of depression (Green *et al.*, 1978; Gold *et al.*, 1980; Petty and Sherman, 1984; Baker and Martin, 1989). Three indices of GABA metabolism have therefore been included in the present study: GABA levels, glutamic acid decarboxylase (GAD) activity and GABA transaminase (GABA-T) activity. Phenelzine (PLZ), tranylcypromine (TCP), imipramine (IMI) and desmethylimipramine (DMI) were chosen for investigation and represent frequently prescribed clinically effective antidepressants. PLZ and TCP are antidepressants that have not previously been analyzed with respect to changes in $GABA_B$ receptor number.

B. BIOCHEMICAL THEORIES OF DEPRESSION

B.1 *Monoamines*

The monoamine deficiency hypothesis (Schildkraut, 1965; Bunney and Davis, 1965) was based mainly on two lines of evidence. First, the clinical observation that drugs that deplete (reserpine) or inhibit the synthesis (α-methyl-p-tyrosine) of amines can produce depressive symptoms. Second, clinically effective antidepressants of the monoamine oxidase (MAO)- inhibiting and tricyclic classes both act to enhance the synaptic availability of the amines noradrenaline and 5-hydroxytryptamine (5-HT). Depression, it was felt, resulted from a functional deficiency of noradrenaline and/or 5-HT at certain central synapses and antidepressants worked by overcoming this deficit.

A great deal of research was initiated as a result of this proposal. In particular, many studies were undertaken to identify deficiencies in the levels of these amines or their metabolites. 3-Methoxy-4-hydroxyphenylethyleneglycol (MHPG), a major noradrenaline metabolite in the central nervous system and 5-hydroxyindole-3-acetic acid (5-HIAA), a 5-HT metabolite, have been extensively studied in the urine, plasma and cerebrospinal fluid of depressed and control subjects (Stahl and Palazidou, 1986). Patients with bipolar depression have been reported to have lower than normal levels of urinary MHPG (Maas *et al.*, 1973; Goodwin and Post, 1975; Edwards *et al.*, 1980). For patients with unipolar depression, low (Maas, 1978), normal (Beckman and

Goodwin, 1980) and even high (Garfinkel *et al.*, 1979) urinary MHPG levels have been found. MHPG levels in the cerebrospinal fluid have been reported to be both decreased (Jimerson *et al.*, 1975) and normal (Shaw *et al.*, 1976; Shopsin *et al.*, 1972) in depression. Jimerson et al. (1975) reported that the acid metabolite of noradrenaline, vanillylmandelic acid (VMA), was also reduced in depression.

As was the case for studies on noradrenaline metabolites, studies on 5-HT metabolites in depressives have also failed to uncover a robust metabolic disturbance Cerebrospinal fluid 5-HIAA levels are reported to be reduced compared to controls in only one-third of the studies (Bunney and Garland, 1981; Baker and Dewhurst, 1985). It has been suggested that this great discrepancy may be due to only certain subgroups of depressives showing altered cerebrospinal fluid 5-HIAA levels (Asberg *et al.*, 1976; van Praag, 1984).

Much less work has been done on 5-HT metabolites in urine and plasma. Nevertheless, there is some evidence for an alteration in the kynurenine pathway [a 5-HT metabolic pathway in humans (Young and Sourkes, 1975)] in depressed patients (Green and Costain, 1979). Ridges (1981) has also suggested that the urinary excretion of 5-HIAA may be used as a guide for selection of antidepressant therapy (Baker and Dewhurst, 1985).

In addition to 5-HT and noradrenaline, a role for dopamine in depression has also been suggested (Randrup and Braestrup, 1977; Jimerson, 1987). Cerebrospinal fluid studies in which probenecid was administered to block transport of acid metabolites, have found decreased concentrations of the dopamine metabolite homovanillic acid (HVA) in depressed patients (Berger *et al.*, 1980). Moreover, low pretreatment levels of HVA in cerebrospinal fluid were predictive of favorable clinical response (Post *et al.*, 1978; Jimerson, 1987). Several "newer" antidepressants have been found that more selectively affect dopamine. Bupropion inhibits dopamine uptake, releases dopamine and acts as a behavioural stimulant (Ferris *et al.*, 1982). Nomifensine also inhibits the uptake of dopamine (noradrenaline to a lesser extent) and produces anti-depressant effects (van Scheylen *et al.*, 1977). One recent avenue of research with drugs that affect dopamine activity (e.g., bupropion) has centered around the possibility of a dopaminergic involvement in certain aspects of depression like anhedonia and psychomotor retardation (Fibiger, 1984).

B.2 *GABA*

There are numerous research findings that support a GABA hypothesis of depression (Bartholini *et al.*, 1985). In an animal model of depression such as learned helplessness induced in rats by exposure to inescapable electric shocks (Seligman and Grove, 1967), the behavioural deficit can be prevented by injection of GABA into the frontal neocortex, lateral geniculate body and hippocampus of the brain (Sherman and Petty, 1982). In the same context, when the GABA antagonist bicuculline is injected into rat hippocampus or cerebrum, a learned helplessness-like state is produced (Petty and Sherman, 1981; Sherman and Petty, 1982; Petty, 1986).

Lower than normal GABA levels have been found in both cerebrospinal fluid (Gold *et al.*, 1980; Gerner and Hare, 1981; Kasa *et al.*, 1982) and plasma (Berrettini *et al.*, 1982; Petty and Sherman, 1982) of depressed patients. This reduction in GABA was not confirmed in a recent study by Korpi *et al.* (1988) in post mortem brain tissue from depressed suicide victims. Reduced activity of the GABA synthetic enzyme glutamic acid decarboxylase (GAD) in the frontal cortex from depressed patients (as compared to controls) was reported by Perry *et al.* (1977). Anticonvulsant drugs such as sodium valporate (Lambert *et al.*, 1975; Emrich *et al.*, 1980) and carbamazepine (Okuma *et al.*, 1973; Ballenger and Post, 1980), both of which have been used in the treatment of lithium-resistant affective disorders, induce a decrease in GABA turnover in rat brain (Bernasconi and Martin, 1979). Electroconvulsive shocks, comparable to electroconvulsive therapy used in the treatment of depression, alter the concentration and synthesis of GABA in rat nucleus accumbens and caudate nucleus when administered chronically (Green *et al.*, 1978). Antidepressant effects have been observed with a diverse range of GABA agonists including progabide, baclofen, fengabine and muscimol in both animal models of depression and in clinical trials (Delina-Stula and Vassout, 1978; Morselli *et al.*, 1980; Lloyd *et al.*, 1983, 1987a,b; Singh *et al.*, 1986). MAO-inhibiting antidepressants such as PLZ, iproniazid and pargyline have been reported to induce an elevation in whole brain GABA levels in rats (Balzer *et al.*, 1960; Popov and Matthies, 1969; Schatz and Lai, 1971; Perry and Hansen, 1973; Patel *et al.*, 1975; Baker *et al.*, 1991; McKenna *et al.*, 1991a).

B.3 *Peptides and the HPA Axis*

In the control and modulation of emotions and motivated behaviours, it is generally accepted that an important interplay exists between the nervous and endocrine systems (Haskett and Rose, 1981). Research on neurophysiological and neuroendocrine functioning has demonstrated that many neural networks involved in changes in behaviour, mood, cognition and perception (Rafuls *et al.*, 1987) are responsive to certain body hormones (Martin *et al.*, 1977). An important component of this neuroendocrine system is the hypothalamus-pituitary-adrenal gland (HPA) axis (Fox, 1984). Several "higher" brain centers, including the basal forebrain, limbic system and midbrain, are involved in regulating HPA function (Stokes and Sikes, 1987).

A link between mental illness and altered HPA function was provided by clinical reports of psychiatric symptoms in patients with primary disorders of the endocrine system (Haskett and Rose, 1981; Leigh and Kramer, 1984). In Cushing's disease, numerous depressive symptoms are frequently seen in the early stages of the disease; these symptoms range from mild dysphoria and overreactions to stress, to dramatically depressed mood with anhedonia, mood-congruent delusions, neurovegetative signs and suicidality (Whybrow and Hurwitz, 1976). Patients with adrenal cortisol deficiency (Addison's disease) also display changes in personality and behaviour (Ettigi and Brown, 1978). Depressive symptoms frequently observed are apathy, loss of interest, fatigue, poverty of thought, irritability and negativism (Rafuls *et al.*, 1987).

With this clinical evidence suggestive of a link between HPA dysfunction and psychiatric symptoms, a considerable amount of research was initiated in an attempt to identify any abnormalities in the levels of the major HPA axis hormones hypothalamic peptide corticotropin-releasing hormone (CRH), anterior pituitary peptide adrenocorticotropic hormone (ACTH), adrenal cortex steroid cortisol and its metabolites 17-hydroxycorticosteroids (17-OHCS) and 17-ketogenic steroids (17-KGS) during depression. In many, but not all, patients with endogenous depression, basal plasma cortisol levels have been found to be elevated over control values (Stokes and Sikes, 1987). Sachar *et al.* (1970) observed that 9 out of 16 depressed patients had elevated cortisol production, which then returned to normal upon recovery. Plasma levels of cortisol and its metabolites have been shown to be elevated during depressed phases and normal during phases of hypomania in bipolar patients (Gibbons and McHugh, 1962; Hullin *et al.*, 1967; Rubin, 1967). Elevated levels of cortisol metabolites have also been found in the urine of depressed patients (Kurland, 1964; Bunney *et al.*, 1967; Mason 1965). Because it has been suggested that urinary levels of 17-OHCS and 17-KGS may not always correlate well with plasma indices of HPA activity (Cope and Black, 1959; Rosner *et al.*, 1963) and likely represent less than 50% of total cortisol secretion (James *et al.*, 1968), several studies have measured urinary free cortisol. In agreement with the metabolite studies, urinary free cortisol is also elevated in many endogenously depressed patients compared to controls and other psychiatric populations (Carroll *et al.*, 1976; Stokes *et al.*, 1984).

Plasma studies on ACTH levels have produced mixed results. Early studies indicated that ACTH levels were not elevated during endogenous depression (Berson and Yalow, 1968) nor was there a difference in ACTH levels between cortisol suppressors and nonsuppressors post-dexamethasone [see below] (Fang *et al.*, 1981; Nasr *et al.*, 1983). More recently, higher than normal levels of ACTH have been found in cortisol nonsuppressors (Reus *et al.*, 1982). This discrepancy in results may be due to plasma sampling time since ACTH is secreted episodically and has a very short half-life (Stokes and Sikes, 1987). The cortisol suppressors/nonsuppressors described above refer to results from the dexamethasone suppression test that has been used in psychiatry to investigate HPA abnormalities in affective disorders (Huang and Mass, 1985). The dexamethasone suppression test was implemented as a diagnostic tool following the observation that a significant proportion of patients with major depressive disorder failed to suppress cortisol secretion after being administered the synthetic corticosteroid dexamethasone (Carroll *et al.*, 1981; Rubin and Marder, 1983).

Measurements of CRH and cortisol levels have also been made in the cerebrospinal fluid. With the use of a radioimmunoassay, elevated levels of immunoreactive CRH have been found in depressed patients compared to normal controls and other patient groups (Nemeroff *et al.*, 1984). It must be noted, however, that no relationship was observed between these cerebrospinal fluid measures and peripheral plasma cortisol measures (Stokes and Sikes, 1987). Results from cerebrospinal fluid cortisol level estimations are consistent with those of other body fluids, i.e. increased cortisol levels in depressed patients compared to normal controls or other psychiatric groups (Carroll *et al.*, 1976; Traskman *et al.*, 1980; Gerner and Wilkins, 1983; Stokes *et al.*, 1984).

An interesting aspect of the involvement of the HPA axis in depression and affective disorders is the involvement of the biogenic amines 5-HT and noradrenaline in the regulation of HPA functioning (Baker and Dewhurst, 1985). Biogenic amines have long been thought of as critically important in both the etiology and pharmacological treatment of depression. In this regard, chronic administration of antidepressants to depressed patients with evidence of HPA hyperactivity typically results in a gradual return to normal HPA activity together with or preceding a return to normal mood (Holsboer *et al.*, 1985; Stokes and Sikes, 1987).

B.4 *Acetylcholine*

A possible role for acetylcholine in the etiology of depression is suggested from the results of a variety of preclinical research studies. Administration of centrally active cholinomimetics (e.g. physostigmine) has been shown to induce behavioural inhibitory effects including lethargy, hypoactivity and decreases in self-stimulation in a variety of animal species (Janowsky *et al.*, 1972a,b). Overstreet *et al.* (1986) have demonstrated that, in rats, the induction of muscarinic acetylcholine receptor up-regulation (due to withdrawal of chronic receptor agonist treatment) is associated with a greater degree of behavioural immobility in a forced swim test, compared to control animals (Janowsky and Risch, 1987). By contrast, anticholinergic drugs reduce behavioural immobility in the forced swim test and are reported to attenuate the effects of inescapable shock [a procedure commonly used to induce learned helplessness (Goldman and Erickson, 1987)]. The forced swim test and learned helplessness are two behavioural paradigms that have been suggested to be animal models of depression (Goldman and Erickson, 1983; McKinney, 1984).

Clinical research studies have also provided evidence for an involvement of acetylcholine in depression. Intravenous administration of physostigmine induces depressive effects on mood (Risch *et al.*, 1980), leads to slowed thoughts and speech and decreased spontaneous behaviour (Davis *et al.*, 1976). An increase in depressive symptoms has been found with manic patients given physostigmine (Janowsky *et al.*, 1973; Davis *et al.*, 1978). In addition, Risch *et al.* (1983) and Nurnberger *et al.* (1981) have observed that administration of the direct cholinergic agonist arecoline to depressed patients induced an increase in depressive symptomology, similar to that previously seen with physostigmine administration.

Further evidence for a role of acetylcholine in depression comes from studies on the influence of acetylcholine on HPA axis function. As noted in section B.3, one characteristic of depression is an alteration in HPA activity including increased cortisol, ACTH and β-endorphin secretion. Several clinical studies (Davis and Davis, 1979; Doerr and Berger, 1983; Risch *et al.*, 1983) have demonstrated significant effects of cholinomimetics on HPA activity in humans. Both physostigmine and arecoline administration significantly increase serum ACTH, cortisol and β-endorphin levels in normal and in psychiatric patients (Janowsky and Risch, 1987). Physostigmine also reverses the dexamethasone-induced suppression of cortisol in normals, an effect which occurs naturally in some depressives (Carroll *et al.*, 1980; Doerr and Berger, 1983).

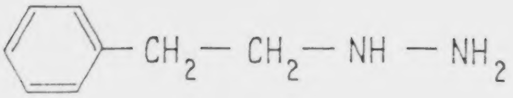
It was proposed by Janowsky and colleagues (Janowsky *et al.*, 1972a) that a balance or interaction exists between adrenergic and cholinergic activity in the regulation of mood. Simply stated, depression was hypothesized to be a syndrome due to a relative excess in central acetylcholine activity compared to normal or decreased noradrenergic and/or dopaminergic activity, and mania was the opposite (Janowsky and Davis, 1979). In support of this hypothesis, it has been observed that methylphenidate (which inhibits noradrenaline and dopamine uptake [Ferris *et al.*, 1972]) -induced psychomotorstimulation in humans is rapidly antagonized by physostigmine. Similarly, physostigmine's inhibitory-depressant effects can be reversed by methylphenidate (Janowsky *et al.*, 1973). In addition, many clinically effective antidepressants from the tricyclic drug class have significant anticholinergic components to their action (Maj *et al.*, 1984).

C. ANTIDEPRESSANT TREATMENTS

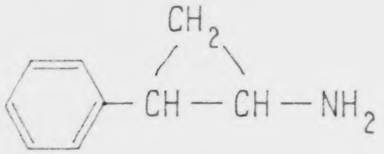
C.1 *Tricyclic Antidepressants*

The use of tricyclic antidepressants began with the landmark clinical work of Roland Kuhn in the late 1950's. In these experiments, he studied the actions of several phenothiazine derivatives in depressed patients and found the iminodibenzyl analogue of promazine, since named IMI, to have substantial antidepressant effects. Tricyclic antidepressants are so named because of their characteristic structure (Fig. 1) which has 3 fused rings containing carbon, hydrogen and nitrogen atoms. The prototypical tricyclic antidepressant is IMI, with other, newer tricyclics having variations in either the central ring structure or the side chain (Lader, 1980) or novel arrangements as found in some of the atypical antidepressants (see below).

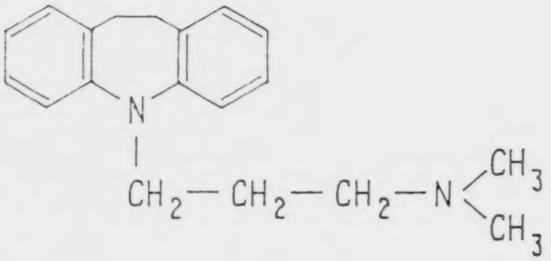
In humans, tricyclics like IMI and amitriptyline are rapidly absorbed and extensively metabolized. Following oral ingestion, absorption is usually complete within 10 h and maximal plasma concentrations obtained after 1-2 h (Lader, 1980). Tricyclic antidepressants are metabolized by four main routes: (1) desmethylation of a side chain,



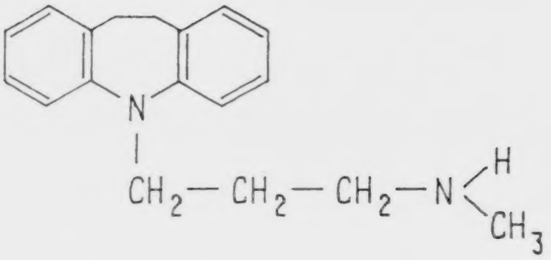
Phenelzine



Tranylcypromine



Imipramine



Desmethylimipramine

Figure 1: Structures of antidepressant drugs used in this thesis

(2) N-oxidation of a side chain, (3) hydroxylation of various positions of the ring structure and (4) glucuronide formation (Rudorfer and Potter, 1985). From these routes many active and inactive metabolites are formed. With IMI, the N-desmethyl, N-oxide and 2-hydroxy metabolites are all active, i.e. they inhibit reuptake of monoamines to some degree.

The N-desmethyl metabolite of IMI, i.e. DMI, is of particular interest because it is itself an antidepressant drug (Mindham, 1979). With the finding that DMI was also present in the body of patients being treated with IMI, it was suggested that perhaps DMI was the active substance producing the antidepressant effects and that the direct administration of DMI might lead to more rapid and enhanced antidepressant effects than those observed with IMI (Mindham, 1979). Several studies have compared DMI with IMI and have failed to indicate a consistent advantage for DMI in either its efficacy or speed of onset (Lafave *et al.*, 1965; Hargreaves and Maxwell, 1967).

Due to the fact that tricyclics are extensively metabolized in the liver, conditions that influence liver function can greatly influence their levels in the body (Dubovsky, 1987). Barbituates, alcohol and anticonvulsants may stimulate liver enzymes, thereby decreasing plasma levels (Rudorfer and Potter, 1985). Conversely, some drugs, e.g., phenothiazines and methylphenidate, compete with the tricyclics for metabolizing liver enzymes, thus slowing the rate of degradation of both compounds (Dubovsky, 1987). As suggested by Baldessarini (1985a), this competition may account for the synergistic action of antipsychotics and stimulants with antidepressants. Additional drug interactions include potentiation of anticholinergic effects of antihistamines and phenothiazines (Sjoqvist, 1965), and a potentially lethal interaction with MAO inhibitors. The pharmacological bases for this toxic MAO inhibitor-tricyclic interaction appears to be excessive central and peripheral concentrations of amines, especially noradrenaline, resulting in pronounced neurotransmitter action (Lader, 1980). The main signs are restlessness, sweating, muscular twitching and rigidity, hyperpyrexia and loss of consciousness (Mindham, 1979).

Side effects associated with tricyclic antidepressant treatment are primarily due to the antagonistic action of tricyclics at both peripheral nervous system and central nervous system muscarinic and α-noradrenergic receptors. Anticholinergic effects include blurred vision, dry mouth, urinary retention, constipation, and excessive sweating (Abramowicz, 1980). Antagonism of α-noradrenergic receptors produces cardiovascular (e.g., hypo/hypertension, tachycardia, arrhythmias) and sedative effects as well as orthostatic hypotension. Photosensitivity and skin rashes have also been reported (Abramowicz, 1980).

C.2 *MAO Inhibitors*

The serendipitous discovery of the mood elevating actions of the MAO-inhibiting antituberculous drug, iproniazid, initiated the use of this class of drug in the treatment of depression (Bosworth, 1959).

MAO is an ubiquitous metabolic enzyme in humans. It is found in most tissues with the exception of the red blood cells and blood plasma (Blaschko, 1952). In the cell, it is localized in the outer membrane of the mitochondria, where it carries out oxidative deamination reactions on a variety of monoamines (Fowler and Ross, 1984). MAO exists in two main forms, MAO-A and MAO-B, which can be distinguished on the basis of substrate preference and selectivity of inhibition (Johnson, 1968). MAO-A preferentially metabolizes 5-HT and noradrenaline and is selectively inhibited by the MAO-inhibitor clorgyline (Johnson, 1968). MAO-B preferentially metabolizes dopamine and β-phenylethylamine and is selectively inhibited by (-)-deprenyl (Johnson, 1968; Finberg and Youdim, 1983).

MAO inhibitors that are currently used or are under study as potential antidepressants can be categorized in two ways: (1) mode of action: irreversible vs. reversible inhibition of MAO (2) inhibition: selective to a given form of MAO or non-selective, inhibiting both MAO-A and -B. The most frequently prescribed MAO-inhibitors (Murphy *et al.*, 1985) and the ones used in the present study, PLZ and TCP, are irreversible nonselective inhibitors of MAO (Tipton and Fowler, 1984).

MAO inhibitors like PLZ and TCP (see Fig. 1) are rapidly absorbed and extensively metabolized within 24 h of their administration (Lader, 1980). Potential routes of metabolism for PLZ include acetylation, deamination (to β-phenylethylamine) and ring hydroxylation (Baker and Coutts, 1989). TCP also undergoes acetylation (Calverly *et al.*, 1981) and ring hydroxylation (Baker *et al.*, 1986) and the breakage of its cyclopropyl ring may occur to potentially yield β-methylphenylethylamine, phenyl-3-n-propylamine and/or amphetamine although this latter route of metabolism remains a matter of debate (Baker and Coutts, 1989).

The most important drug interactions with MAO inhibitors are those involving sympathomimetic amines (Murphy *et al.*, 1985). Examples of sympathomimetic amines are adrenaline, ephedrine and amphetamine. These drug interactions as well as the tyramine "cheese reaction" (see below) can cause hypertension, headaches and sometimes stroke (Sjoqvist, 1965). MAO inhibitors have been shown to prolong or potentiate the effects of many commonly used drugs. The sedative effects of alcohol and the barbituates are enhanced if these drugs are consumed while the patient is on MAO inhibitor therapy (Zemishlany *et al.*, 1983). In addition, a troublesome potentiation of anticholinergic side effects of MAO inhibitors by such drugs as phenothiazines, some antiparkinson drugs and tricyclic antidepressants (Sjoqvist, 1965) has been observed.

MAO inhibitors have been greatly maligned, and their clinical use significantly reduced, because of their reputation for producing unwanted, and at times, dangerous side-effects (Tyrer, 1979). Orthostatic (postural) hypotension is one of the most frequently described peripheral nervous system side effects and is most often a problem in patients with lower pretreatment blood pressure (Murphy *et al.*, 1985).

The second and certainly more severe of the two major peripheral nervous system side effects is the hypertensive crisis. This rapid elevation in blood pressure, also known as the "cheese reaction", can occur when the diet of a patient undergoing MAO inhibitor treatment contains foods that are high in tyramine (Dostert, 1984). The mechanism underlying this "cheese reaction" is most likely the ability of tyramine to act as a sympathomimetic amine and cause release of the elevated stores of noradrenaline (due to MAO inhibition), resulting in an exaggerated effect upon α-adrenergic receptors and dramatic elevation in blood pressure (Marley and Blackwell, 1970; Sandler, 1981). Due to the severity of these reactions, patients are typically given a list of foodstuffs to be avoided or consumed in small quantities. Additional peripheral nervous system side effects seen with MAO inhibitors are blurred vision, constipation and dry mouth (Rabkin *et al.*, 1984).

Central nervous system side effects are also associated with MAO inhibitor treatment. Alterations in the sleep patterns of patients involving cases of insomnia (Murphy *et al.*, 1984) and reductions in the amount of rapid eye movement (REM) sleep (Wyatt *et al.*, 1971) have been observed. Hypomania has been found to occur in 10% of PLZ-treated patients and in approximately 7% of TCP-treated patients (Rabkin *et al.*, 1984).

Several rare side effects due to MAO inhibitor therapy that have been reported include photosensitivity, skin rashes, and hepatotoxicity (Tollefson, 1983; Zemishlany *et al.*, 1983; Murphy *et al.*, 1984; Rabkin *et al.*, 1984). Hepatotoxicity, as indicated above, was a condition that severely limited the early use of MAO inhibitors as antidepressants. However, hepatotoxicity has been reported mostly with iproniazid (Murphy *et al.*, 1985) whereas the currently available MAO inhibitors used in clinical practice [PLZ, TCP, isocarboxazid] (Coutts *et al.*, 1986) appear to be less hepatotoxic (Zisook, 1985).

C.3 *Atypical Antidepressants*

First-generation antidepressants such as the tricyclics and MAO inhibitors described previously have been used clinically for over 30 years (Baldessarini, 1985b). Despite this history of success, their significant side-effect profiles and generally delayed onset of therapeutic action have stimulated research for newer antidepressants with a reduced number of side effects and a more rapid onset of action (Damlouji *et al.*, 1985). The so-called atypical or novel antidepressants have been of particular interest to researchers because they are neither inhibitors of MAO nor, in several cases, potent blockers of uptake of noradrenaline, 5-HT or dopamine (Baldessarini, 1989), and thus their mechanism(s) of action in depression are obscure.

The first of the atypicals to be tested clinically was iprindole. Iprindole has a modified tricyclic structure with an indole group replacing two groups of the standard tricyclic structure (Rudorfer and Potter, 1989), which apparently accounts for its lack of uptake blocking activity. In clinical trials, iprindole has a narrow side effect profile, with mild anticholinergic symptoms, few cases of lethal overdose (Cassidy and Henry, 1987) and a time course for the onset of antidepressant effects similar to that for the tricyclics (Rudorfer and Potter, 1989). The overall clinical efficacy of iprindole in these trials is, however, inconclusive (Baldessarini, 1985b).

Mianserin is a tetracyclic atypical antidepressant that has been used extensively in Europe (Damlouji *et al.*, 1985). Its acute neurochemical activity involves the presynaptic blockade of α_2-noradrenergic receptors, resulting in increased noradrenaline release (Rudorfer and Potter, 1989). The most common side effect of mianserin therapy is sedation (due to strong antihistaminic activity), with few anticholinergic effects reported (Damlouji *et al.*, 1985). In addition, mianserin is reported to have low cardiovascular toxicity and show few interactions with cardiovascular or psychotropic drugs (Conti *et al.*, 1979).

Several other atypicals have been tested for their efficacy as antidepressants. The triazolobenzodiazepines, principally alprazolam and adinazolam, in clinical trials show equivalent antidepressant effects to reference tricyclic antidepressants in major depression, and appear to be particularly useful in depressions associated with anxiety (Rickels *et al.*, 1985; Rudorfer and Potter, 1989). With respect to side effects, alprazolam, for example, has few anticholinergic effects and, due to its benzodiazepine character, has a wide safety margin in overdose (McCormick *et al.*, 1985).

Fluoxetine, which has become one of the most frequently prescribed antidepressants (Medical Letter, 1990) is a selective inhibitor of 5-HT uptake, differing from clomipramine in that its desmethylated metabolite is also a potent and selective 5-HT uptake inhibitor (Benfield *et al.*, 1986). It has been reported to lack anticholinergic, hypotensive and sedative side effects (Schatzberg *et al.*, 1987). Trazodone acts as a serotonergic agonist *in vivo*, and it is thought that m-chlorophenylpiperazine (m-CPP), a major metabolite of this drug, may in fact be the active agent (Potter and Manji, 1990). This novel antidepressant lacks anticholinergic effects but produces considerable sedation (Rudorfer and Potter, 1989).

C.4 *Electroconvulsive Therapy (ECT)*

Convulsive therapies were initiated in the early 1900's as a treatment for schizophrenia due to the supposed mutual antagonism between epilepsy and schizophrenia (Crow and Johnstone, 1979). Subsequent research has shown that epilepsy does not protect against schizophrenia, and ECT is now used infrequently in the treatment of schizophrenia (Dubovsky, 1987). ECT has, however, been used successfully in the treatment of depression for over 40 years (Baldessarini, 1985b). The neurological basis for the effectiveness of ECT in depression is at present unknown, but a number of neurochemical changes following ECT treatment have been established. Animal studies have found several changes in both the 5-HT and noradrenaline neurotransmitter systems including increased 5-HT brain levels following single or multiple ECT treatments and increased noradrenaline turnover in discrete brain regions (Schildkraut *et al.*, 1967; Essman, 1973). ECT-induced changes in the number and function of biogenic amine and GABA receptors (see section D) have also been reported. For a comprehensive review of neurotransmitter systems affected by ECT see Lerer (1987).

Clinical observations following ECT indicate that, as with MAO-inhibitor and tricyclic antidepressant treatment, significant improvements are seen in such diverse functions as sleep, appetite and sex drive (Feldman and Quenzer, 1984). Confusion and memory loss are the major neurological side effects of ECT treatment (Dubovsky, 1987). These effects are typically of short duration following treatment, with little evidence available for their persistence beyond 6 months post treatment (Weiner, 1984).

The use of ECT in the clinical setting is usually restricted to severely depressed or suicidal patients who have not responded to earlier antidepressant drug therapies (Dubovsky, 1987). In addition, ECT has been used in the treatment of manic or catatonic patients, and, because there are few absolute contraindications to ECT [e.g., increased intracranial pressure] (Bernstein, 1983), it is seen as a safe form of treatment in patients that are at high risk of toxicity from antidepressant drugs [especially elderly patients, pregnant women and patients with severe cardiovascular disease] (Baldessarini, 1985b).

D. RECEPTOR CHANGES ASSOCIATED WITH LONG TERM ANTIDEPRESSANT TREATMENTS

A great deal of research effort has been expended over the last 40 years in an attempt to identify the mechanism(s) of action of antidepressant treatments. In the early period of this research the main area of study was an analysis of the acute effects of antidepressant drugs on brain neurochemistry. This preoccupation with acute drug effects was based on the strongly held belief in the monoamine deficiency hypothesis of depression, and more importantly, in the proposed alleviation of depression by the uptake-blocking or enzyme-inhibiting actions of the classical antidepressants. Several observations suggested that this theory was too simplistic to account for the therapeutic action of antidepressants: [1] there is a 2-3 week delay in clinical response after initiation of treatment despite almost immediate drug effects on uptake and MAO activity (Lapierre, 1985); [2] cocaine is a potent blocker of noradrenaline uptake, yet is not an effective antidepressant (Post *et al.*, 1974); [3] atypical antidepressants, such as iprindole and mianserin, do not have the same acute neurochemical effects as the classical antidepressants but are still efficacious antidepressants (Baldessarini, 1989). Because of these observations and the finding that chronic antidepressant therapy is associated with several emergent changes in monoamine receptors (Sugrue, 1983), much current research investigating the mechanism(s) of action of antidepressant treatments is focussed on the chronic effects of these treatments on various brain receptor systems.

In subsequent sections, D.1-D.6, details will be given about antidepressant-induced changes in the number of several neurotransmitter receptor systems and their associated cellular components. Although a detailed discussion about cellular signaling is beyond the scope of this thesis, a brief description about receptors and receptor-activated transduction pathways is provided for clarity.

Receptors are membrane proteins found on the outer (extracellular) surface of plasma membranes, which serve as targets for extracellular signal molecules, like neurotransmitters (Darnell *et al.*, 1986). Formation of a receptor-signal molecule complex, initiates a transduction pathway whereby an external signal, carried by the signal molecule, is transduced into an internal signal, carried by an ion or second messenger, which ultimately regulates one or more cellular processes (Berridge, 1985). The mechanisms of action of receptors for a number of different hormones and neurotransmitters have suggested that these receptors could be grouped into families based on utilization of the same transduction pathway (Dixon *et al.*, 1988). The two receptor families of most relevance to this discussion are (1) receptors linked to ion channels (e.g. $GABA_A$/benzodiazepine) and (2) receptors linked to second messenger production through guanine nucleotide binding regulatory proteins (G-proteins) [e.g. β-adrenoceptors, 5-HT receptors]. The two most common G-protein-linked second messenger systems are the adenylate cyclase - cyclic adenosine monophosphate (cAMP) system and the phosphodiesterase - diacylglycerol, Ca^{2+}, inositol triphosphate (IP_3) system.

D.1 β-*Adrenoceptors*

Adaptive changes in the number and function of noradrenaline β-adrenoceptors are the most commonly reported emergent changes. Foremost among these is a desensitization of the β-adrenoceptor-coupled adenylate cyclase system (Sulser, 1987). Sulser and colleagues (Vetulani and Sulser, 1975; Vetulani *et al.*, 1976a,b) were the first to demonstrate that long-term treatment with DMI or iprindole and repeated electroconvulsive shocks reduced the noradrenaline-stimulated cyclic adenosine monophosphate (cAMP) accumulation in rat limbic forebrain slices. Subsequent research confirmed this finding in rat limbic forebrain and in cerebral cortex following repeated administration of a variety of antidepressant drugs: DMI, IMI, amitriptyline, clomipramine, zimelidine, pargyline, TCP, mianserin and nisoxetine (Schultz, 1976; Frazer and Mendels, 1977; Schmidt and Thornberry, 1977; Fraser *et al.*, 1978; Mishra and Sulser, 1978; Wolfe *et al.*, 1978; Korf *et al.*, 1979; Mishra *et al.*, 1979, 1980; Maj *et al.*, 1984; Sulser, 1987). Biochemical explanations for the antidepressant-induced subsensitivity include increased synaptic cleft noradrenaline levels, changes in β-adrenoceptor density (see below), alterations in phosphodiesterase activity, changes in coupling factors and modification of properties of the cellular membrane (Sugrue, 1983). At present it is not known which, if any, of these possibilities are correct, but the finding that iprindole and zimelidine, two drugs that do not alter noradrenaline uptake (Gluckman and Baum, 1969; Ross *et al.*, 1976) yet still induce subsensitivity of the β-adrenoceptor-adenylate cyclase system, argues against the increased synaptic noradrenaline hypothesis. A mediation through reduced β-adrenoceptor density also appears unlikely based on the ability of mianserin and nisoxetine to down-regulate adenylate cyclase function without changing the number of β-adrenoceptors (Sugrue, 1983).

Using radioligand binding studies, it has been shown that chronic administration of tricyclics, MAO inhibitors, novel antidepressants and repeated electroconvulsive shocks induces a reduction in the number (density) of β-adrenoceptor binding sites in rat cortical tissue (Banerjee *et al.*, 1977; Wolfe *et al.*, 1978; Sellinger-Barnette *et al.*, 1980; Maggi *et al.*, 1980; Peroutka and Snyder, 1980; Kellar *et al.*, 1981a; Sugrue, 1982). These decreases are not accompanied by any significant effect on the affinity of β-adrenoceptors (Maj *et al.*, 1984) for the radioligand. As described above, one effective antidepressant, mianserin, fails to reduce β-adrenoceptor binding (Mishra *et al.*, 1980; Charney *et al.*, 1981). Despite this exception, the generality of the binding reduction and the fact that it only occurs following repeated, and not acute, antidepressant treatment (Heninger and Charney, 1987) supports the importance of this effect in the action of antidepressants. Moreover, non-antidepressant drugs such as most antipsychotics and anxiolytics, are ineffective in reducing β-adrenoceptor binding (Charney *et al.*, 1981).

Alterations in β-adrenoceptors have also been demonstrated electrophysiologically and with *in vivo* behavioural paradigms. Using the inhibitory response to microiontophoretically applied noradrenaline as a dependent variable, the responsiveness of β-adrenoceptors is diminished following repeated treatment with most antidepressants (Olpe and Schellenberg, 1980; Aghajanian, 1981; Schultz *et al.*, 1981). Olpe and Schellenberg (1980) found that chronic administration of DMI, clomipramine, maprotiline, or TCP significantly reduced the responsiveness of cingulate cortical neurons to noradrenaline. In the cerebellum, another brain region where the response to noradrenaline is thought to be mediated by β_2-adrenoceptors, Siggins and Schultz (1979) observed that chronic DMI decreased the sensitivity of Purkinje cells to noradrenaline. Similarly, Huang (1979) found that repeated DMI treatment enhanced the spontaneous firing rate of noradrenaline-sensitive hippocampal pyramidal cells, suggesting that the inhibitory effect of noradrenaline was reduced (Maj *et al.*, 1984). Several other authors have been unable to identify any antidepressant effects on hippocampal cell functioning. Repeated treatment with a variety of antidepressants (DMI, IMI, clomipramine, amitriptyline, zimelidine and iprindole) did not alter the inhibitory response to noradrenaline (de Montigny and Aghajanian, 1978; Gallager and Bunney, 1979; de Montigny *et al.*, 1981; Maj *et al.*, 1984). The exact reason for the discrepancy between these hippocampal results and those from the cortex and cerebellum is unknown, although the noradrenaline response has been shown to vary with the brain region under investigation (Blier and de Montigny, 1984).

With the *in vivo* behavioural approach, one way of assessing antidepressant-induced changes in receptor function is to measure changes in the behavioural effects of an administered drug thought to act through the receptor(s) of interest (Baker and Greenshaw, 1989). It has been previously shown that salbutamol, a β_2-adrenoceptor agonist (Brittain *et al.*, 1968), induces hypoactivity in animals (Przegalinski *et al.*, 1980; Mogilnicka, 1986). Salbutamol-induced hypoactivity has thus been used as an *in vivo* measure of β_2-adrenoceptor function. With this approach, Przegalinski *et al.* (1983, 1984) reported that salbutamol-induced hypoactivity was reduced by chronic administration of the antidepressant drugs IMI, DMI, amitriptyline, fluvoxamine, citalopram, mianserin and nialamide.

D.2 α-*Adrenoceptors*

Electrophysiological and behavioural studies indicate that the sensitivity of α_1-adrenoceptors is enhanced by chronic antidepressant administration (Sugrue, 1983; Maj *et al.*, 1984). Repeated treatment with IMI, DMI, clomipramine, amitriptyline or iprindole enhance the response to iontophoretically applied noradrenaline in the rat facial motor nucleus (an area rich in α_1-receptors) [Menkes *et al.*, 1980; Menkes and Aghajanian, 1981]. Additionally, the response of lateral geniculate neurons to iontophoretically applied noradrenaline is also enhanced by chronic treatment with IMI, DMI, amitriptyline or iprindole (Menkes and Aghajanian, 1981). In high doses, clonidine elicits aggressive behaviour in mice, which may be mediated by postsynaptic

α_1-adrenoceptors (Morpurgo, 1968; Maj *et al.*, 1980). Following chronic administration of a number of antidepressant drugs, this aggressive behaviour is increased (Maj *et al.*, 1980, 1982). Despite the above functional evidence for supersensitivity of α_1-adrenoceptors, the majority of receptor binding studies have not found a change in α_1-binding following long-term antidepressant administration (Bergstrom and Kellar, 1979; Rosenblatt *et al.*, 1979; Snyder and Peroutka, 1982; Menkes *et al.*, 1983; Palfreyman *et al.*, 1986). The reason for this lack of a consistent binding effect is unknown, but it has been suggested that radioligand choice and a brain region selectivity for receptor changes might be complicating factors (Maj *et al.*, 1984; Baker and Greenshaw, 1989).

Based on electrophysiological and behavioural experiments, α_2-adrenoceptor sensitivity is reduced following chronic antidepressant treatments. Clonidine-induced motor suppression is attenuated in rats repeatedly treated with IMI, DMI, maprotiline, clorgyline, PLZ, TCP or electroconvulsive shocks (Delini-Stula, 1978; Spyraki and Fibiger, 1980; Heal *et al.*, 1981; Cohen *et al.*, 1982a; Passarelli and Scotti de Carolis, 1982; Greenshaw *et al.*, 1988; McKenna *et al.*, 1991b). Clonidine, which in low doses decreases the firing of locus ceruleus cells in control animals (Maj *et al.*, 1984), has no such effect on rats chronically pretreated with IMI, DMI or zimelidine (Svensson and Usdin, 1978; Scuvée-Moreau and Svensson, 1982).

There is a great deal of inconsistency in reports on the effects of chronic antidepressants on α_2-adrenoceptor binding. Depending upon the length of the treatment period, increases (4-7 d), no effect (14-21 d) or decreases (21-28 d) in the density of [^{3}H]-clonidine binding to α_2-adrenergic binding sites have been observed (Johnson *et al.*, 1980; Askura *et al.*, 1982; Cohen *et al.*, 1982b; Pilc and Vetulani, 1982; Sugrue, 1982). The early increase in α_2 binding likely only represents some early adaptive change and is not directly reponsible for the antidepressant effect typically observed only after longer periods of drug administration (Maj *et al.*, 1984). Reisine *et al.* (1982) suggest that initial changes in α_2-adrenoceptors may be responsible for the down-regulation of β-adrenoceptors also seen following chronic antidepressant drug treatments.

D.3 *5-HT Receptors*

In parallel with the studies on adrenergic receptors, a significant amount of work has been carried out assessing the effects of chronic antidepressant treatments on 5-HT receptors. With an electrophysiological paradigm, Blier, de Montigny and others have demonstrated that antidepressants of different classes all enhance 5-HT neurotransmission, but do so via different mechanisms (Blier *et al.*, 1990). Tricyclic antidepressants administered for 14 d increased the effectiveness of 5-HT pathway stimulation on the firing activity of postsynaptic neurons in the amygdala and hippocampus (Wang and Aghajanian, 1980; Blier *et al.*, 1987; de Montigny *et al.*, 1989), dorsal and ventral lateral geniculate nucleus (de Montigny and Aghajanian, 1978) and the somatosensory cortex (Jones, 1980). The underlying neurological basis for this effect appears to be a sensitization of postsynaptic 5-HT_{1A} receptors (de Montigny, 1984; de Montigny *et al.*, 1989). Consistent with this view is the observation that the responsiveness of hippocampus pyramidal neurons to microiontophoretically applied 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) [a 5-HT_{1A} agonist] is enhanced following long-term tricyclic treatment (de Montigny *et al.*, 1989). In addition to chronic tricyclic antidepressant treatment, chronic mianserin (Blier *et al.*, 1984) and repeated ECT administration (de Montigny *et al.*, 1989) also induce a sensitization of postsynaptic receptors to 5-HT.

Chronic administration of MAO inhibitors enhances 5-HT transmission by increasing the availability of releasable 5-HT (Blier *et al.*, 1990). In a study with the MAO-A selective inhibitor clorgyline and the nonselective MAO-inhibitor PLZ, both antidepressants were found to increase the effectiveness of stimulation of the raphé-hippocampus 5-HT pathway (Blier *et al.*, 1986). Of interest, in the clorgyline group there was also a decrease in the responsiveness of the postsynaptic hippocampal neurons to 5-HT, yet the net effect on the 5-HT pathway was enhanced. It appears that a presynaptic effect of MAO-A inhibition can overcome the decreased responsiveness of postsynaptic neurons to 5-HT (Blier *et al.*, 1990). More recently, Bouthillier *et al.* (1989) observed that the β_2-adrenoceptor agonist flerobuterol induces an enhancement of 5-HT transmission similar to that induced by the MAO inhibitors. This result is in accord with previous reports of antidepressant efficacy for two other β_2-agonists salbutamol and clenbuterol (Lecrubier *et al.*, 1980; Lerer *et al.*, 1981; Simon *et al.*, 1985). According to Blier *et al.* (1990), the finding of Bouthillier *et al.* that a β_2-adrenoceptor agonist enhances 5-HT neurotransmission is also important because it suggests that this class of drugs might exert their antidepressant effect through the 5-HT system and that an enhancement of the releasable 5-HT pool can be achieved in several ways.

Radioligand binding studies on 5-HT receptor subtypes following chronic antidepressant treatments have yielded variable results. In general, the number of 5-HT_2 binding sites is reduced following repeated antidepressant drugs (Peroutka and Snyder, 1980; Kellar *et al.*, 1981b; Zsilla *et al.*, 1983; Scott and Crews, 1986) and increased by repeated ECT (Green *et al.*, 1983; Kellar and Bergstrom, 1983). The number of 5-HT_1 binding sites using [^{3}H]-5-HT as a radioligand has been found to be reduced or unchanged following repeated administration with various antidepressant treatments (Charney *et al.*, 1981; Blier *et al.*, 1990). Following 14 d tricyclic antidepressant treatment, an increase in the number of 5-HT_{1a} binding sites (as labelled with 8-OH-DPAT) has been found in several rat brain regions including hippocampus, septum and cerebral cortex (Welner *et al.*, 1989).

Variable results have also been obtained for antidepressant effects on 5-HT-coupled processes and behavioural measures of 5-HT function. A decrease in 8-OH-DPAT-induced hypothermia (a behavioural measure of 5-HT$_{1a}$ receptor function) has been found following chronic administration of several antidepressant treatments, including electroconvulsive shocks (Goodwin *et al.*, 1985, 1987). Chronic amitriptyline and mianserin enhance the behavioural response of rats to 5-hydroxytryptophan (Mogilnicka and Klimek, 1979). Friedman *et al.* (1983) observed an enhanced head-twitch response induced by the direct postsynaptic agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) in rats treated chronically with tricyclic antidepressants. The head-twitch response is believed to be mediated by 5-HT receptors on motorneurons (Jacobs and Klemfuss, 1975). Stolz and Marsden (1982) reported potentiation of the 5-MeODMT "serotonin syndrome" [hind limb abduction, forepaw treading, lateral head weaving, straub tail] (Maj *et al.*, 1984) in rats treated repeatedly with amitriptyline. In contrast, chronic DMI, zimelidine or mianserin has also been found to decrease the 5-MeODMT-induced head-twitch reaction in mice (Fuxe *et al.*, 1982; Blackshear and Sanders-Bush, 1982). Similarly, Lucki and Frazer (1982) observed that the MAO inhibitors nialamide, pargyline and PLZ, given repeatedly, prevent the lysergide- or 5-MeODMT-induced serotonin syndrome.

5-HT receptors have been shown to be coupled to both adenylate cyclase activity and inositol phosphate metabolism (Sanders-Bush and Conn, 1987). An effect of antidepressants on the inositol phosphate response was first described by Kendall and Nahorski (1985). These authors found a 58% and a 67% decrease in 5-HT-stimulated inositol phosphate formation after chronic IMI and iprindole respectively. Newman and Lerer (1988a) noted a similar reduction in 5-HT-induced accumulation of inositol monophosphate, inositol biphosphate, inositol triphosphate after repeated DMI treatment. A lack of an effect of repeated electroconvulsive shocks on 5-HT-coupled processes has also been observed (Godfrey *et al.*, 1987; Newman *et al.*, 1987). Godfrey *et al.* (1987) found no effect of chronic electroconvulsive shocks, zimelidine or 5,7-dihydroxytryptamine lesions on 5-HT-stimulated inositol phosphate formation, but did find a 33% decrease in this 5-HT response following chronic DMI.

Modification of the 5-HT-coupled adenylate cyclase system also appears to occur following chronic antidepressant treatments. Recent work from Newman and Lerer (1988b) found that chronic electroconvulsive shocks or DMI showed no effect on 5-HT-stimulated activity in hippocampal membranes, but did induce a significant decrease in the degree of inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT. These authors suggested that these findings may provide a biochemical correlate for the decrease in 8-OH-DPAT-induced hypothermia (a behavioural measure of 5-HT_{1a} receptor function) previously observed by Goodwin *et al.* (1985, 1987) [Newman and Lerer, 1989].

It has recently been suggested that the 5-HT_3 receptor may also be a target for antidepressant drugs and may play a role in their mechanisms of action. Schmidt and Peroutka (1989) reported that several antidepressant drugs including 5-HT uptake inhibitors such as chlorimipramine, sertraline, paroxetine and fluoxetine, exhibit nanomolar affinity for [^{3}H]-quipazine-labelled 5-HT_3 binding sites in rat cortical membranes. This 5-HT_3 receptor-antidepressant drug interaction is controversial as Hoyer *et al.* (1989) did not find a nanomolar affinity of 5-HT uptake inhibiting antidepressants for 5-HT_3 receptors in membranes prepared from either rat entorhinal cortex or the neuroblastoma cell line N1E-115.

D.4 *Dopamine Receptors*

Several reports provide evidence for a modification of dopamine receptors in the action of antidepressant treatments (Spyraki and Fibiger, 1981; Sugrue, 1983).

Chronic administration of antidepressants induces subsensitivity of presynaptic dopamine receptors (Serra *et al.*, 1979; Nielsen, 1986). Repeated treatment with DMI or nomifensine also reduces the number of presynaptic dopamine binding sites, as labelled by [^{3}H]-dopamine in the striatum (Lee and Tang, 1982). By comparison using behavioural tests, it has been shown that repeated treatment with DMI, iprindole and electroconvulsive shocks induced a supersensitivity of postsynaptic dopamine receptors (Grahame-Smith *et al.*, 1978; Spyraki and Fibiger, 1981). Plaznik and Kostowski (1987) observed that repeat treatment with DMI, citalopram or electroconvulsive shocks enhanced the behavioural effects of dopaminergic agonists injected into the nucleus accumbens. A supersensitivity of dopamine receptors was also identified by Smialowski and Bijack (1986) using an *in vitro* paradigm. These authors found that 14 d treatment with IMI enhanced the firing rate of hippocampal slice preparations following dopamine application. In a recent radiolabelled binding study, Klimek and Nielson (1987) found that chronic administration of various antidepressants significantly reduced the number of dopamine D_1 binding sites (measured with [^{3}H]-SCH-23390), but did not alter the number of dopamine D_2 binding sites.

In addition to the dopamine D_1 and D_2 subtypes mentioned above, three new subtypes designated D_3, D_4, D_5 have been isolated (Sokoloff *et al.*, 1990; Van Tol *et al.*, 1991; Sunahara *et al.*, 1991). At present, no studies have been conducted to determine the effects of chronic antidepressant treatments on their number or function.

D.5 *Acetylcholine Receptors*

Changes in the number and function of brain acetylcholine receptors have been analyzed following chronic antidepressant treatments. Using radioligand binding assays, Rehavi *et al.* (1980) and Goldman and Erickson (1983) reported that chronic amitriptyline administration increased the number of muscarinic acetylcholine receptors in the pons medulla and hippocampus brain regions in the mouse and the cerebral cortex of the rat, respectively. Similarly, Koide and Matsushita (1981) found increased muscarinic acetylcholine receptor binding in the rat striatum following repeated treatment with IMI and DMI. Repeated electroconvulsive shocks have also been reported to alter the number of muscarinic acetylcholine receptors in several rat brain regions including an increase in receptor number in the cerebral cortex (Gulati *et al.*, 1982) and a decrease in receptor number in the hippocampus (Dashieff *et al.*, 1982). Several other studies have not been able to confirm these effects of antidepressant treatments on mucarinic receptor number. Peroutka and Snyder (1980) and Maggi *et al.* (1980) found no effect of the chronic administration of a wide variety of antidepressant drugs on the binding to muscarinic acetylcholine receptors in either the cerebral cortex or striatum. Likewise, Deakin *et al.* (1981) and Kellar *et al.* (1981a) found no change in the number of rat brain muscarinic receptors following repeated electroconvulsive shocks.

Fewer studies have looked at changes in acetylcholine receptor function after prolonged antidepressant treatment. Using an electrophysiological approach, Menkes and Aghajanian (1981) reported no effect of the chronic treatment with IMI, DMI, clomipramine, amitriptyline or iprindole on the responsiveness of lateral geniculate nucleus neurons to microiontophoretically applied carbachol. In contrast, Newman and Lerer (1988b) reported a decrease in the degree of inhibition of forskolin-stimulated adenylate cyclase activity by carbachol after chronic electroconvulsive shocks or DMI, thus indicating a decrease in acetylcholine receptor function.

D.6 *GABA$_A$/Benzodiazepine and GABA$_B$ Receptors*

One non-monoamine receptor system currently receiving increased attention is the GABAergic system. Results from research in this area, although far from unequivocal, are suggestive of an alteration in GABA$_A$/benzodiazepine and/or GABA$_B$ receptors in the mechanism of action of antidepressant treatments. Suzdak and Gianutsos (1985a) have reported that long-term administration of IMI or nomifensine produced a significant reduction in the density of GABA$_A$ binding sites in mouse cerebral cortex and hippocampus. Consistent with this GABA$_A$ reduction is the finding that chronic treatment with different classes of antidepressants (DMI, zimelidine, bupropion, adinozolam and maprotiline) also significantly reduced the number of benzodiazepine binding sites in rat brain (Suranyi-Cadotte *et al.*, 1984; Barbaccia *et al.*, 1986). A recent report by Kimber *et al.* (1987) failed to replicate these earlier findings. They observed that 21 d administration of DMI, TCP and zimelidine did not significantly alter either the number or affinity of benzodiazepine binding sites as measured with [^{3}H]-flunitrazepam. A recent and extensive report by Squires and Saederup (1988) found that 23 clinically effective antidepressants fully or partially reversed the inhibitory action of GABA on [^{3}H]-*t*-butylbicyclophosphorothionate (TBPS) binding, an effect consistent with the interaction of antidepressants with GABA$_A$/benzodiazepine receptors.

Several functional measures of GABA$_A$/benzodiazepine receptor activity are altered by chronic antidepressant treatment. Repeated (18 d) treatment of rats with IMI significantly reduced the ability of GABA to stimulate ^{36}Cl$^-$ uptake into cerebral cortical membrane vesicles, without altering the basal ^{36}Cl$^-$ uptake (Fernandez-Teruel *et al.*, 1989). Bouthillier and de Montigny (1987) observed that 21 d administration of DMI, trimipramine and citalopram to rats reduced the effect of flurazepam application on cholecystokinin (CCK)-induced activation of hippocampal pyramidal cells. These authors concluded that the results were consistent with an antidepressant-induced down-regulation of brain benzodiazepine receptors. Decreased $GABA_A$/benzodiazepine receptor function was also suggested from the results of Borsini *et al.* (1986), who found THIP-induced antinociception was reduced by chronic, but not acute, DMI administration.

$GABA_B$ receptors have also been reported to be modified by chronic antidepressant treatments. Lloyd *et al.* (1985, 1989) found that a diverse group of antidepressant drugs (DMI, amitriptyline, maprotiline, viloxazine, zimelidine, fluoxetine, citalopram, progabide, fengabine, mianserin, trazodone, pargyline) as well as repeated electroconvulsive shocks, all induced a significant increase in the number of $GABA_B$ binding sites in rat frontal cortex. Because this effect was induced by antidepressants from all major drug classes (MAO inhibitors, tricyclics, novel) and electroconvulsive shocks, they postulated that an increase in the number of $GABA_B$ binding sites was a common mechanism in the action of antidepressant treatments. Subsequent research both with receptor binding assays and functional tests has not provided conclusive evidence for this proposal. Suzdak and Gianutsos (1986) found that long-term treatment of mice with IMI induced an increase in the number of $GABA_B$ binding sites in the cerebral cortex and also led to an increase in the baclofen potentiation of noradrenaline-stimulated cAMP accumulation in cortical slices. Similarly, Gray and Green (1987) observed that 14 d treatment with DMI, amitriptyline, mianserin, zimelidine and electroconvulsive shocks significantly enhanced the ability of baclofen to inhibit K^+-evoked release of 5-HT from mouse frontal cortex slices. Moreover, these same antidepressants also enhanced the baclofen-induced hypothermic response, consistent with an increase in $GABA_B$ receptor function (Gray *et al.*, 1987). In contrast, Borsini *et al.* (1986), using baclofen-induced antinociception as an *in vivo* measure of

$GABA_B$ receptor function, found no effect of chronic DMI on this response. Long-term treatment with DMI and IMI also failed to alter $GABA_B$ receptor-coupled signal transduction as monitored by the ability of (-)-baclofen to inhibit the forskolin-stimulated adenylate cyclase activity in rat frontal cortex membranes (Szekely *et al.*, 1987). Szekely *et al.* (1987) also analyzed the effect of the antidepressants on $GABA_B$ receptor binding. An increase in the number of $GABA_B$ binding sites was found using [^{3}H]-GABA in the binding assay. When [^{3}H]-baclofen was used as the radioligand, no effect of the antidepressants on $GABA_B$ binding sites was observed. [^{3}H]-Baclofen binding to $GABA_B$ sites has, however, recently been shown to be increased in the hippocampus following chronic treatment with lithium and carbamazepine (Motohashi *et al.*, 1989). Cross and Horton (1987, 1988) have not found any effect on [^{3}H]-$GABA_B$ binding in rat frontal cortex or whole cortex following 21 d administration of DMI or zimelidine. This drug administration protocol did, however, produce a significant decrease in the number of 5-HT_2 binding sites.

Based on the experimental data presented above, it is evident that chronic antidepressant treatments induce changes in the number and function of a variety of neurotransmitter receptors. In the present study, it was of interest to attempt to replicate and extend the reported antidepressant drug-induced changes in GABA receptor binding, and to determine their functional significance with an *in vivo* behavioural test. For comparative purposes parallel changes in β-adrenoceptors were also measured. β-Adrenoceptors were chosen for study because, as outlined in Section D.1, a decrease in the number and subsensitivity in the function of β-adrenoceptors is a common emergent feature following chronic antidepressant administration. The next two sections (E and F) provide a brief overview of the characteristics of both noradrenergic and GABAergic neurons. For noradrenergic receptors only β-adrenoceptor subtypes were included, while for GABA receptors both $GABA_A$ and $GABA_B$ are discussed.

E. NORADRENALINE

E.1 *Synthesis, Storage, Release and Uptake*

The synthesis of noradrenaline occurs via the catecholamine biosynthetic pathway. This pathway contains 5 primary enzymes: tyrosine hydroxylase; aromatic *L*-amino acid decarboxylase; dopamine β-hydroxylase; pteridine reductase; and phenylethanolamine-N-methyltransferase, that convert sequentially the amino acid precursor tyrosine, into the catecholamines dopamine, noradrenaline and adrenaline (Fig. 2). Catecholamines derive their name from their characteristic catechol nucleus (a 3,4-dihydroxylated benzene ring) [Fig. 2]. The first, and rate-limiting enzyme in the pathway, tyrosine hydroxylase, converts tyrosine to *l*-3,4-dihydroxyphenylalanine (*l*-dopa). Tyrosine hydroxylase is present in all neurons producing catecholamines and requires molecular oxygen (O_2), Fe^{2+} and a tetrahydropteridine cofactor for activity (Nagatsu *et al.*, 1964). The tetrahydropteridine cofactor will be regenerated by a pteridine reductase (not shown in Fig 2). *L*-dopa is next decarboxylated to dopamine by the pyridoxal phosphate-dependent enzyme aromatic amino acid decarboxylase (McGeer *et al.*, 1978). Dopamine is then hydroxylated by dopamine β-hydroxylase to form noradrenaline. Dopamine β-hydroxylase, like tyrosine hydroxylase, is a mixed function oxidase that requires molecular O_2 and utilizes ascorbic acid as a cofactor (Cooper *et al.*, 1986). Dopamine β-hydroxylase is also a Cu^{2+}-containing enzyme, allowing for enzyme inhibition by such Cu^{2+} chelating agents as disulfiram and fuscaric acid (McGeer *et al.*, 1978). The last enzyme in the catecholamine synthetic pathway is phenylethanolamine-N-methyltransferase. This enzyme converts noradrenaline to adrenaline using S-adenosylmethionine as methyl group donor. Not all cells that secrete catecholamines express all five of these biosynthetic enzymes. In noradrenergic cells,

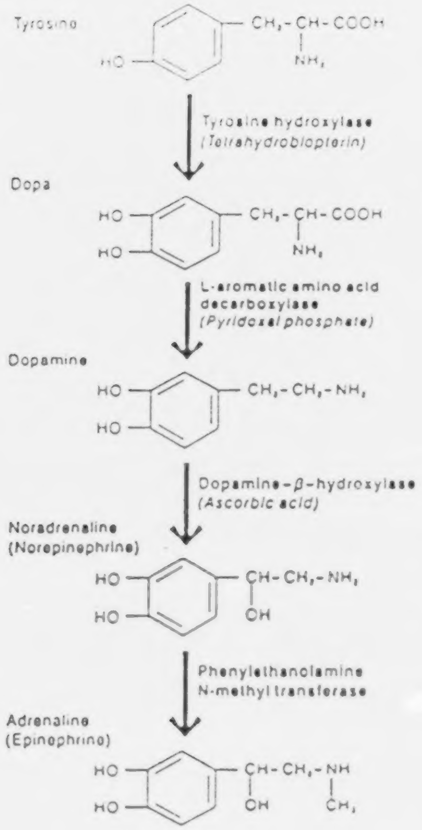

Tyrosine
HO
$CH_2-CH-COOH$
NH_2
Tyrosine hydroxylase
(Tetrahydrobiopterin)
Dopa
HO
HO
$CH_2-CH-COOH$
NH_2
L-aromatic amino acid
decarboxylase
(Pyridoxal phosphate)
Dopamine
HO
HO
$CH_2-CH_2-NH_2$
Dopamine-β-hydroxylase
(Ascorbic acid)
Noradrenaline
(Norepinephrine)
HO
HO
$CH-CH_2-NH_2$
OH
Phenylethanolamine
N-methyl transferase
Adrenaline
(Epinephrine)
HO
HO
$CH-CH_2-NH$
OH
CH_3

Figure 2: Catecholamine biosynthetic pathway. Reprinted with permission by M. Lader and The Upjohn Company from *Introduction to Psychopharmacology* (1980).

there is no expression of the methyltransferase enzyme and in dopaminergic cells neither the methyltransferase nor dopamine β-hydroxylase is expressed (Kandel and Schwartz, 1985).

In the presynaptic terminal, synthesized noradrenaline is stored within highly specialized granular particles or vesicles. These vesicles also contain adenosine nucleotides, primarily ATP, a protein called chromogranin, thought to be involved in the storage process, and dopamine β-hydroxylase (McGeer *et al.*, 1978). The concentration of catecholamines like noradrenaline in these vesicles has been estimated to be 0.6 M - more than twice the osmolarity of mammalian body fluid. It has been suggested that some form of salt linkage between the anionic phosphate groups of ATP and the amine group of noradrenaline must exist to allow for such high concentrations of noradrenaline to exist without the vesicles becoming hyperosmotic and bursting (McGeer *et al.*, 1978).

Experimental studies from both peripheral and central nervous system noradrenergic neurons indicate that the primary mechanism for release of noradrenaline is through exocytosis triggered by a depolarization of the nerve terminal and subsequent influx of Ca^{2+} ions (Douglas, 1968; Raiteri *et al.*, 1975; Colburn *et al.*, 1976). A second release mechanism independent of exocytosis has been demonstrated for noradrenaline release caused by sympathomimetic amines (Raiteri *et al.*, 1977). By this process, sympathomimetic amines induce a release of noradrenaline from the storage vesicles into the axoplasma of the neuron followed by a carrier-mediated release into the synapse (Baker and Dyck, 1985). Following release, the vast majority of synaptic noradrenaline is inactivated by a high affinity, Na^{+}-dependent uptake process back into the presynaptic terminals (Paton, 1980). Data indicate that as much as 80% of released noradrenaline is taken up by this process, while the remainder is metabolized, or diffuses away from the synapse and is taken up by other cells (McGeer *et al.*, 1978).

The two major enzymes of importance in the metabolism of noradrenaline are MAO and catechol-O-methyltransferase (COMT). Figure 3 outlines the metabolic routes utilizing these enzymes, and the resultant metabolites. Which metabolites are formed differs between the peripheral and central nervous systems. In the periphery, the aldehyde formed by the action of MAO is preferentially converted to its corresponding acid, while in the central nervous system, the aldehyde is primarily reduced to an alcohol. Thus, the principle metabolites of peripheral metabolism are normetanephrine (NM), 3,4-dihydroxymandelic acid (DMA), and vanillylmandelic acid (VMA), and from central metabolism 3,4-dihydroxyphenylglycol (DOPEG) and 3-methoxy-4-hydroxyphenylglycol (MHPG) [McGeer *et al.*, 1978).

E.2 *β-Adrenoceptor Subtypes:* β_1 *and* β_2

The existence of distinct subtypes of β-adrenoceptors was firmly established by Lands and co-workers (Lands *et al.*, 1967a,b). These researchers demonstrated that the rank order of potency of a series of catecholamines (isoproterenol, noradrenaline and adrenaline) for several β-adrenergic receptor-mediated responses could be divided into two major groups, so-called β_1 and β_2, reflecting the existence of two types of β-adrenoceptors (Minneman *et al.*, 1981). β-Adrenoceptors mediating cardiac stimulation, fatty acid mobilization from adipose tissue, and inhibition of small intestine contraction were stimulated by the catecholamines with a rank order of potency of isoproterenol > adrenaline = noradrenaline. These were called β_1-receptors. β-Receptors mediating bronchodilation, vasodilation, inhibition of uterine contraction, and contraction of the KCl-relaxed diaphragm were stimulated by the catecholamines with the rank order of potency of isoproterenol > adrenaline >> noradrenaline. These

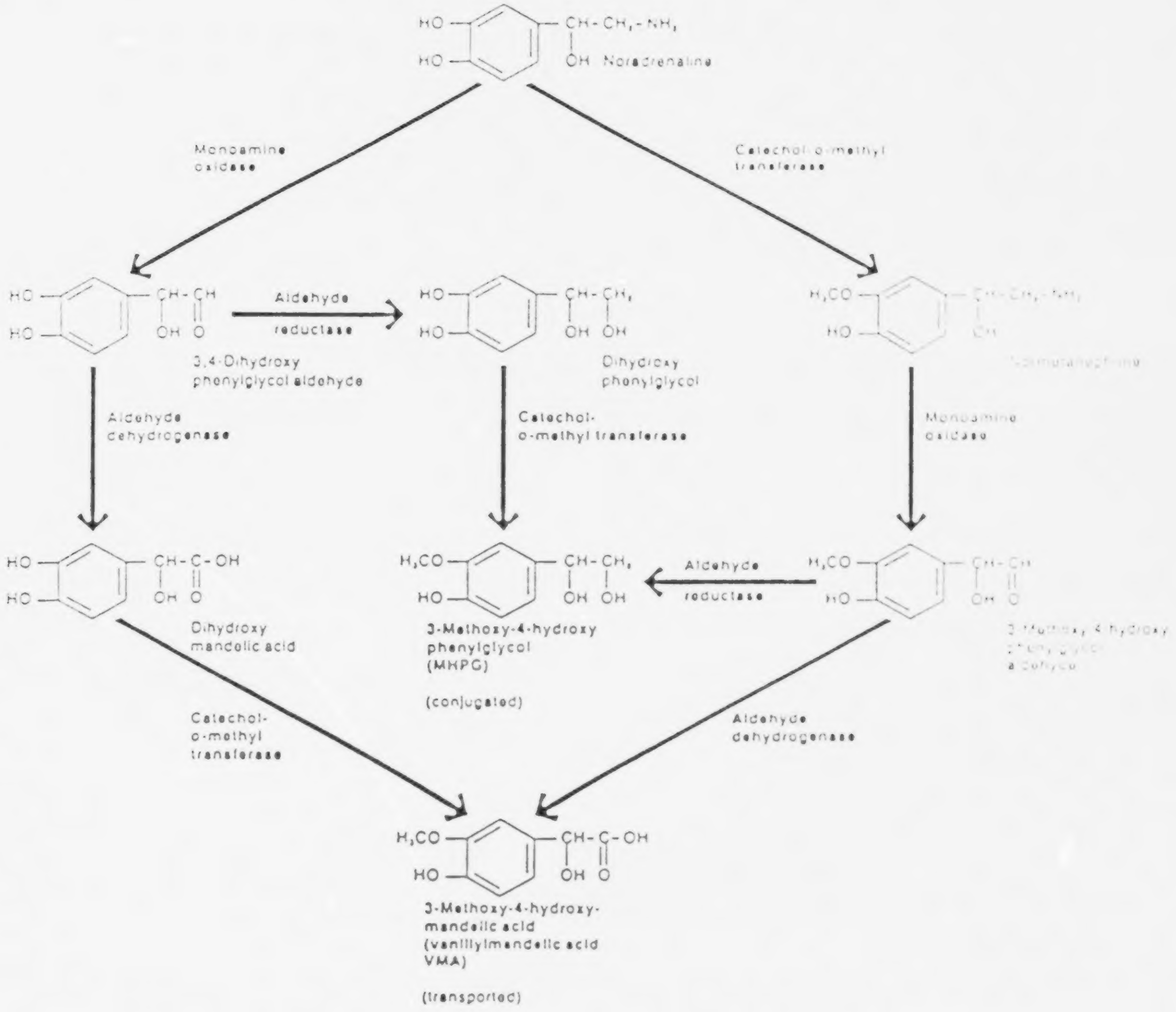

HO
HO
CH-CH$_2$-NH$_2$
OH
Noradrenaline
Monoamine oxidase
Catechol-o-methyl transferase
HO
HO
CH-CH
OH O
3,4-Dihydroxy phenylglycol aldehyde
Aldehyde reductase
HO
HO
CH-CH$_2$
OH OH
Dihydroxy phenylglycol
H$_3$CO
HO
CH-CH$_2$-NH$_2$
OH
Normetanephrine
Aldehyde dehydrogenase
Catechol-o-methyl transferase
Monoamine oxidase
HO
HO
CH-C-OH
OH O
Dihydroxy mandelic acid
H$_3$CO
HO
CH-CH$_2$
OH OH
3-Methoxy-4-hydroxy phenylglycol (MHPG)
(conjugated)
Aldehyde reductase
H$_3$CO
HO
CH-CH
OH O
3-Methoxy-4-hydroxy phenylglycol aldehyde
Catechol-o-methyl transferase
Aldehyde dehydrogenase
H$_3$CO
HO
CH-C-OH
OH O
3-Methoxy-4-hydroxy-mandelic acid (vanillylmandelic acid VMA)
(transported)

Figure 3: Catabolic routes for noradrenaline metabolism. Shown are enzymes involved and principal metabolites. Reprinted with permission by M. Lader and The Upjohn Company from *Introduction to Psychopharmacology* (1980).

were called β_2-receptors (Minneman *et al.*, 1981). Subsequent research confirmed the existence of β-adrenoceptors with different pharmacological properties in these and other peripheral tissues (Arnold and McAuliff, 1969; O'Donnell and Wanstall, 1979).

With the use of radioligand binding and autoradiographic techniques, the existence and distribution of β-adrenoceptor subtypes (β_1 and β_2) in mammalian brain has also been determined. In rat brain, high total β-adrenoceptor numbers are found in the superficial layers of the neocortex, in the caudate-putamen, nucleus accumbens, olfactory tubercles, substantia nigra, nucleus interpeduncularis, subiculum and pia mater (Palacios and Kuhar, 1982). Brain regions containing lower receptor numbers include the cerebellum, hippocampus, thalamus, hypothalamus, amygdala, brainstem and medulla (Palacios and Kuhar, 1982). Throughout these brain regions, β_1-adrenoceptors are the major subtype, except in the cerebellum, where β_2-adrenoceptors are predominant (U'Prichard *et al.*, 1978; Minneman *et al.*, 1979; Nahorski, 1981; Janowsky and Sulser, 1987). In addition, within a given brain region β_1- and β_2-adrenoceptors are heterogeneously distributed. Rainbow *et al.* (1984) have shown that layers I-III, V and VI of the cerebral cortex and certain thalamic areas (such as the gelatinosus, ventroposterior and dorsal lateral geniculate nuclei) are specifically enriched in β_1-adrenoceptors. Conversely, layer IV of the cerebral cortex and other thalamic areas (such as the lateral posterior, paraventricular, and reticular nuclei) are specifically enriched in β_2-adrenoceptors (Wolfe, 1991). Interestingly, the localization of β-adrenoceptors in brain does not show a consistently high correlation with measurements of presynaptic noradrenergic innervation (Minneman *et al.*, 1981; Janowsky and Sulser, 1987). Thus, high numbers of β-adrenoceptors are found in the caudate-putamen, olfactory tubercles and substantia nigra, yet these areas contain only low densities of noradrenergic terminals (Swanson and Hartman, 1975; Moore and Bloom, 1979; Palacios and Kuhar, 1982).

In brain, both β_1- and β_2-adrenoceptors are coupled to the adenylate cyclase - cAMP generating system, the activation of which results in increased levels of the intracellular second messenger cAMP (Janowsky and Sulser, 1987). In accord with such an association, guanine nucleotides have been shown to markedly alter the binding characteristics of β-adrenoceptors. Guanine 5′-triphosphate (GTP), an essential cofactor in the stimulation of adenylate cyclase activity (Rodbell, 1980) reduces the affinity of agonists, but not antagonists, for β-adrenoceptors (Maguire *et al.*, 1976; Lefkowitz *et al.*, 1976). Guanine nucleotides also reduce the proportion of the agonist-occupied receptors in the high agonist-affinity state and, at maximal concentrations, convert all of the agonist-occupied receptors to a lower-affinity state (Kent *et al.*, 1980; Janowsky and Sulser, 1987). Concomitant with this shift to a lower affinity is a nucleotide-mediated increase in adenylate cyclase catalytic activity (Londos *et al.*, 1974; Maguire *et al.*, 1977).

Molecular biological techniques have been used successfully in the identification and characterization of β-adrenoceptor subtypes. The genes encoding the β_1- and β_2-adrenoceptors have been isolated (Frielle *et al.*, 1987; Emorine *et al.*, 1987). These genes are believed to belong to a family of homologous genes that encode for integral membrane receptor proteins (Dixon *et al.*, 1988) which contain several membrane-spanning domains and are coupled to regulatory G proteins (Caron and Lefkowitz, 1991). In addition to the β_1 and β_2 subtypes, a third subtype, a so-called β_3-adrenoceptor, has recently been identified. Emorine *et al.* (1989) have isolated a cDNA that encodes for this third β-adrenoceptor subtype in the human genome. In parallel with the β_1 and β_2 subtypes, the encoded β_3-receptor contains the classic features of a G-protein-coupled receptor, including the seven membrane spanning domain arrangement (Caron and Lefkowitz, 1991). The amino acid sequence of the β_3-receptor is approximately 50% identical to that of the human β_1- or β_2-adrenoceptors. Expression of the

β_3-adrenoceptor in eukaryotic cells revealed that it has different pharmacological properties to those of the β_1- or β_2-adrenoceptors. In particular, only 2 of 11 classical β-adrenoceptor antagonists sufficiently inhibited a noradrenaline- or adrenaline-stimulated accumulation of cAMP in cells expressing the β_3-receptor. Moreover, the compound BRL 37344, which has been suggested to be a potent agonist at the β_3-adrenoceptor in guinea pig ileum (Bond and Clarke, 1988), was among the most effective β-adrenoceptor agonists (at inducing cAMP accummulations) in β_3-adrenoceptor expressing cells, whereas it was less efficient in β_2-adrenoceptor expressing cells and almost ineffective in β_1 expressing cells (Emorine *et al.*, 1989). The functional significance of this β_3-adrenoceptor has not been clearly demonstrated, but it has been suggested, based on drug studies conducted in animals, that β_3-adrenoceptors may mediate the sympathetic control of various metabolic processes in the digestive tract, adipose tissue and skeletal muscle (Emorine *et al.*, 1989).

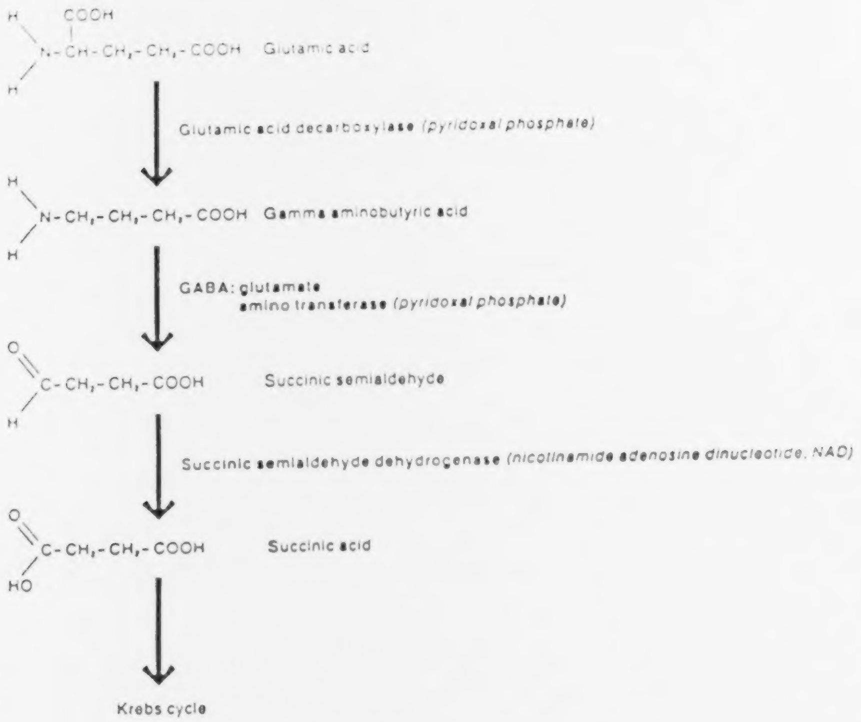
F. γ-AMINOBUTYRIC ACID (GABA)

First identified as a constituent of mammalian brain in the 1950s, GABA has since been shown to function as an important inhibitory neurotransmitter (Roberts *et al.*, 1976). Electrophysiological and radioligand binding studies reveal that GABA-responsive sites exist in virtually all areas of the central nervous system (Enna, 1983). It is estimated that in 10 to 40% of all synaptic terminals in the hippocampus, cerebral cortex and substantia nigra GABA is involved in neurotransmission (Iversen and Bloom, 1972; Schon and Iversen, 1974). Dysfunction of the GABAergic system has been implicated in the etiology of a number of disease states including tardive dyskinesia (Fibiger and Lloyd, 1984), epilepsy (Morselli and Lloyd, 1983), Huntington's chorea (Perry *et al.*, 1973; Bird and Iversen, 1974), Parkinson's disease (Lloyd and Hornykiewicz, 1975) and depression (Morselli *et al.*, 1981).

F.1 *Synthesis, Storage, Release and Uptake*

The majority of GABA found in the brain is synthesized from glutamic acid by the enzyme glutamic acid decarboxylase [GAD] (Yoneda and Roberts, 1982) [Fig. 4]. GAD is a pyridoxal phosphate-dependent enzyme that is expressed exclusively in GABA neurons and is used as a specific GABAergic nerve terminal marker (Feldman and Quenzer, 1984). GABA is catabolized by another pyridoxal phosphate-dependent enzyme, GABA transaminase (GABA-T) to form succinic semialdehyde. Succinic semialdehyde is converted subsequently to succinic acid by succinic semialdehyde dehydrogenase. GABA-T is located in axon terminals, postsynaptic structures and in glial cells (Feldman and Quenzer, 1984). GABA concentrations at nerve terminals range from 50 to 150 mM (Fonnum and Walger, 1973), with most of the GABA thought to be sequestered in vesicles (Fonnum, 1985). The glutamic acid used for the synthesis of GABA is primarily derived from glucose or glutamine (Shank and Campbell, 1982; Fonnum, 1985). With the dependence on glucose as a glutamic acid source, GABA synthesis is intimately linked to carbohydrate metabolism (Cooper *et al.*, 1982). By way of a GABA "shunt", α-ketoglutaric acid is shunted away from the Krebs cycle to provide the precursor glutamic acid for the synthesis of GABA. Moreover, the succinic acid formed from GABA degradation will re-enter the Krebs cycle (Fig. 5).

Studies *in vitro* have shown that GABA is released from brain slices or synaptosomes by a Ca^{2+}-dependent process (Iversen *et al.*, 1971; Ryan and Roskoski, 1975). Recent reports indicate that a Ca^{2+}-independent mechanism may also be involved in GABA release (Bernath *et al.*, 1989; Bernath and Zigmond, 1990) but its role under physiological conditions is still to be determined. Following release, GABA is taken up into nerve terminals and glia by a Na^{+}-dependent high affinity uptake system (Iversen and Kelly, 1975). Lower affinity GABA uptake systems have also been demonstrated



Glutamic acid
Glutamic acid decarboxylase (pyridoxal phosphate)
Gamma aminobutyric acid
GABA: glutamate amino transferase (pyridoxal phosphate)
Succinic semialdehyde
Succinic semialdehyde dehydrogenase (nicotinamide adenosine dinucleotide, NAD)
Succinic acid
Krebs cycle

Figure 4: Synthesis and breakdown of GABA. Reprinted with permission by M. Lader and The Upjohn Company from *Introduction to Psychopharmacology* (1980).

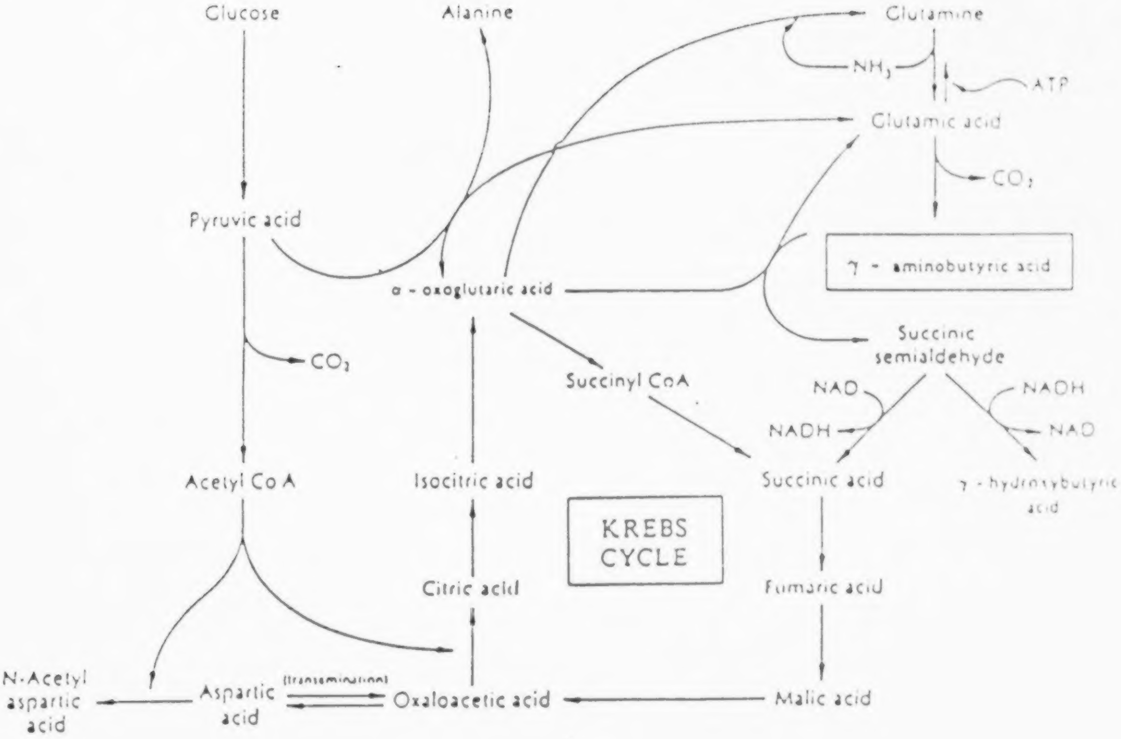

Glucose
Alanine
Glutamine
NH$_3$
ATP
Glutamic acid
CO$_2$
Pyruvic acid
γ - aminobutyric acid
α - oxoglutaric acid
Succinic semialdehyde
CO$_2$
Succinyl CoA
NAD
NADH
NADH
NAD
Acetyl Co A
Isocitric acid
Succinic acid
γ - hydroxybutyric acid
KREBS CYCLE
Citric acid
Fumaric acid
N-Acetyl aspartic acid
Aspartic acid
(transamination)
Oxaloacetic acid
Malic acid

Figure 5: Metabolism of GABA and its inter-relationship with carbohydrate metabolism. Reprinted with permission by Oxford University Press from *The Biochemical Basis of Neuropharmacology* (1986), J.R. Cooper, F.E. Bloom and R.H. Roth, p. 128.

(Levi and Raiteri, 1973; Wood and Sidhu, 1986). Uptake of GABA into glial cells serves an important role in the recycling of GABA. In glia, the NH_3 produced by the action of GABA-T on GABA is combined with α-ketoglutarate to form glutamic acid. Because glia lack the GAD enzyme, the glutamic acid is converted to glutamine which is then transported across to the axon terminals where it will be converted to form an additional source of glutamic acid for GABA synthesis (Reubi *et al.*, 1978).

F.2 *$GABA_A$/Benzodiazepine Receptor Complex*

Electrophysiology, pharmacology and radioligand binding studies have all been used in the identification and characterization of GABA receptors (Enna, 1983; Krogsgaard-Larsen, 1988). GABA receptors can be divided into 2 principal classes: bicuculline-sensitive [$GABA_A$] (Curtis *et al.*, 1974) and bicuculline-insensitive [$GABA_B$] receptors (Hill and Bowery, 1981; Bowery, 1983). The regional distribution of $GABA_A$ receptors in brain is shown in Table 1. The greatest concentration is found in the cerebellum, with the cerebral cortex and hippocampus having the next highest concentrations. The thalamus, amygdala and caudate nucleus have intermediate levels, while the rest of the basal ganglia, lower cerebellar nuclei and medulla have the lowest concentrations of $GABA_A$ binding sites (Enna, 1983).

Radioligand binding studies of $GABA_A$ receptors have been carried out with a variety of receptor agonists, including isoguvacine (Morin and Wasterlain, 1980), muscimol (Dufreudis, 1980), 4,5,6,7-tetrahydroisoxazolo-5,4-c)-pyridin-3-ol [THIP] (Falch and Krogsgaard-Larsen, 1982), piperidine-4-sulfonic acid (Krogsgaard-Larsen *et al.*, 1981) and GABA (Olsen *et al.*, 1981). All ligands tested exhibited at least biphasic binding kinetics, revealing both high- and low-affinity binding sites (Johnston, 1986; Olsen, 1981). An "ultra" low-affinity $GABA_A$ site has also been suggested based on

	pmol/g wet wt	
Brain Region	$GABA_A$	$GABA_B$
Olfactory bulb		
Internal granule layer	43.83 ± 3.08	9.21 ± 0.34
External plexiform layer	54.76 ± 3.75	15.43 ± 1.45
Glomerular layer	23.95 ± 0.23	21.04 ± 3.26
Olfactory nerve layer	15.12 ± 2.18	9.06 ± 0.81
Frontal cortex	63.68 ± 4.49	31.73 ± 4.09
Anterior olfactory nucleus	23.8 ± 4.05	26.49 ± 0.49
Basal ganglia		
Caudate putamen	9.73 ± 2.22	4.86 ± 0.05
Nucleus accumbens	9.2 ± 1.43	4.58 ± 0.62
Medial septum	9.45 ± 2.93	6.49 ± 2.46
Globus pallidus	3.05 ± 0.27	9.76 ± 2.83*
Anterior commissure	5.06 ± 0.92	2.95 ± 0.10
Frontal motor cortex	18.55 ± 4.45	14.09 ± 0.37
Frontal somatosensory cortex	17.04 ± 3.23	11.71 ± 0.21
Temporal cortex auditory area	13.33 ± 0.44	19.49 ± 3.21*
Corpus collosum	3.10 ± 0.02	3.42 ± 0.27
Dentate gyrus molecular layer	19.8 ± 1.73	15.5 ± 1.7
Dentate gyrus granular layer	13.5 ± 1.2	8.4 ± 0.9
Hippocampus		
Stratum oriens	14.9 ± 1.3	10.1 ± 1.1
Stratum pyramidal	16.2 ± 1.4	8.4 ± 0.9
Stratum radiatum	15.9 ± 1.4	12.7 ± 1.4
Thalamus		
Laterodorsal nucleus	18.28 5.05	14.48 ± 0.84
Ventrolateral nucleus	14.18 ± 0.19	16.18 ± 3.09
Ventromedial nucleus	17.73 ± 1.78	17.68 ± 3.38
Rhomboid nucleus	13.63 ± 0.39	8.85 ± 1.32
Medial ventroposterior nucleus	15.94 ± 0.85	21.87 ± 4.16
Lateral ventroposterior nucleus	27.27 ± 1.36	19.37 ± 1.23
Lateral posterior	18.81 ± 0.43	27.3 ± 2.76*
Dorsal lateral geniculate	21.83 ± 11.29	24.72 ± 2.52
Ventral lateral geniculate	7.85 ± 0.49	8.97 ± 0.90
Medial geniculate	30.49 ± 3.09	26.13 ± 5.79
Lateral amygdaloid nucleus	11.25 ± 0.24	25.45 ± 1.44*
Medial habenula nucleus	6.39 ± 0.55	13.36 ± 1.29*
Lateral habenula nucleus	11.84 ± 1.76	9.76 ± 0.59
Interpeduncular nucleus	8.09 ± 0.59	33.62 ± 1.72*
Superior colliculus	13.89 ± 0.53	22.63 ± 3.90*
Brachium	11.32 ± 1.02	12.89 ± 1.35
Superficial gray layer	19.49 ± 22.94	20.81 ± 4.48
Optic nerve layer	14.04 ± 1.97	11.13 ± 0.87
Intermediate gray layer	13.64 ± 1.29	9.12 ± 0.63
Substantia nigra pars campacta	5.78 ± 1.50	4.79 ± 0.80
Substantia nigra pars reticulata	20.22 ± 3.18	10.65 ± 0.68
Dorsal raphé nucleus	17.73 ± 0.26	11.25 ± 0.35
Cerebellum		
Granule cell layer	59.60 ± 5.68	16.8 ± 5.18
Molecular layer	11.39 ± 0.98	49.08 ± 1.93*
White matter layer	5.75 ± 0.79	7.47 ± 1.56

Table 1: Regional distribution of $GABA_A$ and $GABA_B$ receptors in rat brain. * Region in which the concentration of $GABA_B$ sites was significantly ($p \leq 0.01$) higher than that of $GABA_A$ sites. Reprinted with permission from *Neuroscience, Volume 20*, N.G. Bowery, A.L. Hudson and G.W. Price, $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. Copyright 1987, Pergamon Press plc.

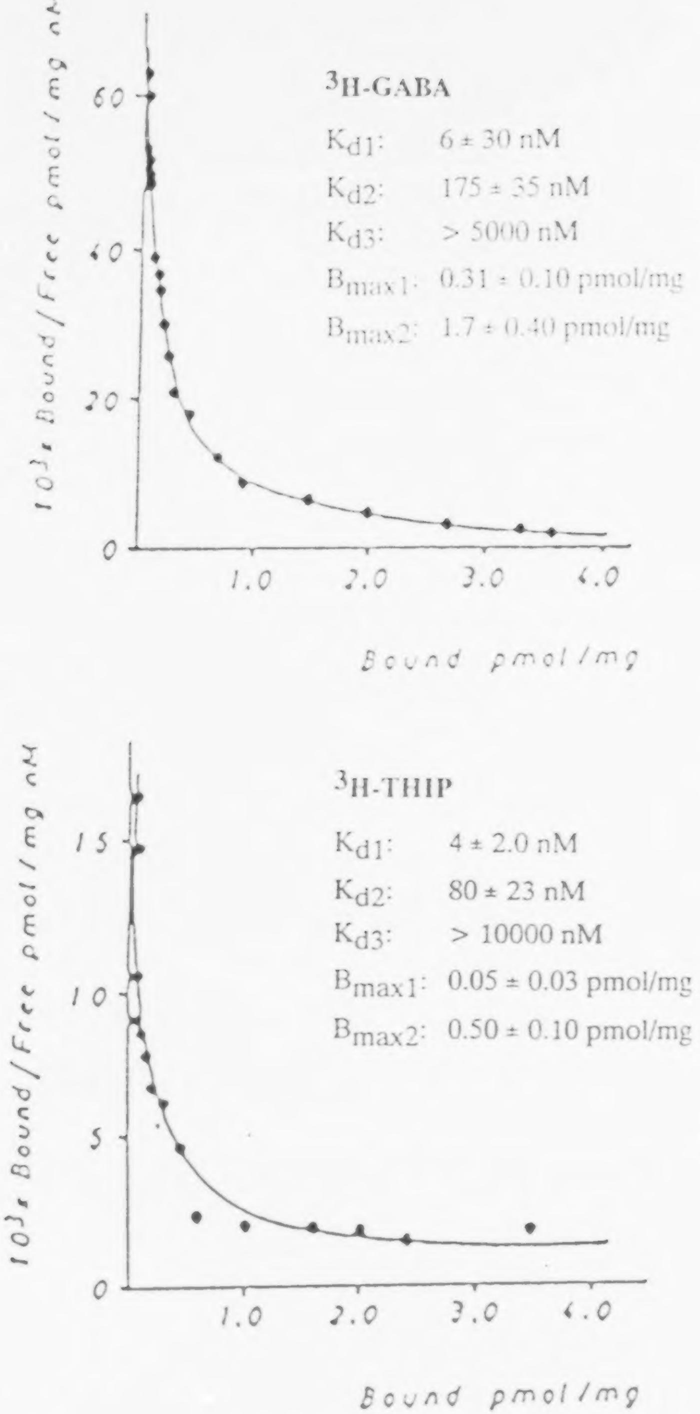
work principally with [^{3}H]-THIP (Falch and Krogsgaard-Larsen, 1982; Olsen and Snowman, 1982). Table 2 displays the affinity states for GABA$_A$ receptors and Fig. 6 gives an example of a GABA$_A$ receptor binding estimation.

GABA$_A$ receptors are coupled to a Cl$^-$ ion channel whose activation results in a net influx or efflux of Cl$^-$ ions, dependent upon the cellular concentration gradient (Enna and Gallagher, 1983). Activation of presynaptic GABA$_A$ receptors leads to a net efflux of Cl$^-$ ions, causing a partial depolarization of the cell (Enna and Defrance, 1980). This effect is usually referred to as presynaptic inhibition since the amount of transmitter released from the partially depolarized terminal is reduced (Enna, 1983). GABA$_A$-mediated presynaptic inhibition occurs in both the brain [regulation of the evoked release of glutamic acid and dopamine] (Bowery, 1983) and spinal cord [at the terminals of 1_a primary afferent fibers] (Haefely and Polc, 1986). By contrast, activation of postsynaptic GABA$_A$ receptors, causes an influx of Cl$^-$ ions, hyperpolarizing the cell and decreasing its sensitivity to excitatory inputs (Krogsgaard-Larsen, 1988). The end result of activation of both types of receptors is the same: an inhibitory effect at the cellular level.

A facilitation of GABAergic neurotransmission has been implicated in the actions of benzodiazepines for some time (Costa *et al.*, 1974; Haefely *et al.*, 1975). The exact site of this effect remained unknown until the discovery of high affinity binding sites for benzodiazepines in brain synaptic membranes (Mohler and Okada, 1977; Squires and Braestrup, 1977). Subsequent localization of these binding sites to synapses known to be GABAergic (Braestrup *et al.*, 1979) and a near perfect overlapping of GABA$_A$ and benzodiazepine receptors based on monoclonal antibody studies (Schoch *et al.*, 1985) suggested a close physical association. Moreover, Tallman *et al.* (1978) and Martin and Candy (1978) had previously demonstrated that GABA enhanced the affinity of benzodiazepines for their receptors in synaptic preparations. Definitive proof

Receptor population	Equilibrium binding constant (K_d, nM)	Associated ion(s)	Cellular response
GABA$_A$ (Bicuculline-sensitive, baclofen-insensitive)			
High affinity	3-10	Chloride	Hyperpolarization (brain)
Low affinity	50-120		Partial depolarization (spinal cord)
Ultralow affinity	1,000-10,000		
GABA$_B$ (Bicuculline-insensitive, baclofen-sensitive)			
High affinity	30-60	Calcium	Inhibition of transmitter release Potentiation of transmitter-stimulated cAMP production
Low affinity	100-300	Potassium	Hyperpolarization

Table 2: Classification and properties of GABA receptors. Reprinted with permission by John Wiley and Sons, Inc. from *Benzodiazepine/GABA Receptors and Chloride Channels: Structural and Functional Properties*, R.W. Olsen and J.C. Venter (eds.), Copyright 1986 by Alan R. Liss, Inc.



^{3}H-GABA

K_{d1}: 6 ± 30 nM
K_{d2}: 175 ± 35 nM
K_{d3}: > 5000 nM
B_{max1}: 0.31 ± 0.10 pmol/mg
B_{max2}: 1.7 ± 0.40 pmol/mg

^{3}H-THIP

K_{d1}: 4 ± 2.0 nM
K_{d2}: 80 ± 23 nM
K_{d3}: > 10000 nM
B_{max1}: 0.05 ± 0.03 pmol/mg
B_{max2}: 0.50 ± 0.10 pmol/mg

Figure 6: Scatchard plots of the specific binding of radioactive GABA and THIP to rat brain $GABA_A$ binding sites. Binding parameters are derived from computer-fitted nonlinear regression analysis. Reprinted with permission by John Wiley and Sons, Inc. from *Benzodiazepine/GABA Receptors and Chloride Channels: Structural and Functional Properties*, R.W. Olsen and J.C. Venter (eds.), Copyright 1986 by Alan R. Liss, Inc.

for their association was provided by co-purification (Stephenson *et al.*, 1982) and co-immunoprecipitation (Schoch *et al.*, 1985) and recent molecular cloning (Pritchett *et al.*, 1989) of protein complexes containing binding sites for both GABA and benzodiazepines (reviewed by Haefely, 1989).

A current model for the interaction of GABA and benzodiazepines is shown in Fig. 7. In this GABA/benzodiazepine receptor complex, benzodiazepine receptor ligands can allosterically modulate GABA-mediated chloride channel openings in a positive [+] (agonists) or negative [-] (inverse agonist) fashion (Costa, 1985). Benzodiazepine receptor agonists appear to increase the frequency of channel openings (Study and Barker, 1982). Receptor purification and molecular cloning experiments indicate the $GABA_A$/benzodiazepine receptor complex to be an integral multimeric protein structure composed of 2 or 3 subunits designated α, β and γ (Schofield *et al.*, 1989; Pritchett *et al.*, 1989). Traditional stoichiometry for the site was $\alpha_2\ \beta_2$ but with firm identification of the γ subunit by Pritchett *et al.* (1989) a pentameric structure appears more likely (Costa, 1989; Sieghart, 1989). Heterogeneity exists within the subunits themselves, with multiple forms of the α and β subunits having been established (Barnard *et al.*, 1989). Based on photoaffinity labelling experiments, the α subunit appears to contain the benzodiazepine recognition site, since it binds $[^3H]$-flunitrazepam, while the β-subunit contains the $GABA_A$ recognition site using $[^3H]$-muscimol as the label (Casalotti *et al.*, 1986). Autoradiographic studies with a variety of ligands including $[^3H]$-GABA, $[^3H]$-bicuculline, $[^3H]$-muscimol and $[^3H]$-flunitrazepam (Young and Kuhar, 1980; Palacios *et al.*, 1981; Unnerstal *et al.*, 1981; Olsen *et al.*, 1984; Richards *et al.*, 1984; Bowery *et al.*, 1987) indicate that benzodiazepine binding site distribution correlates well with that of the low affinity $GABA_A$ binding site.

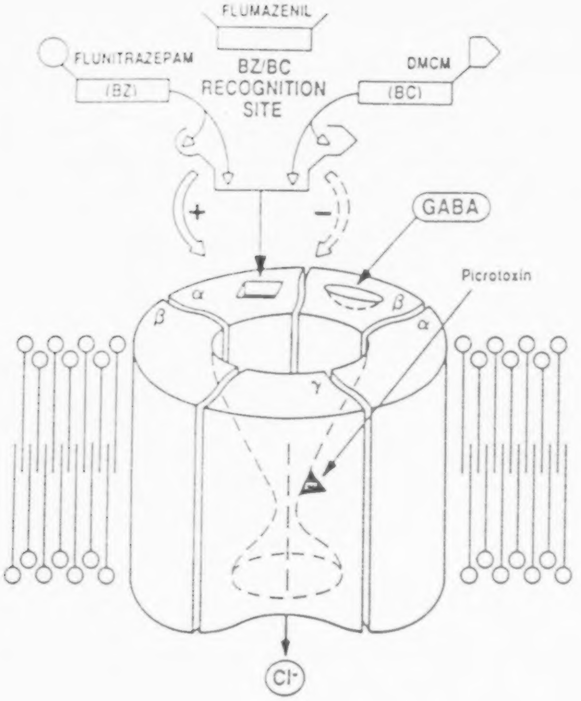

FLUMAZENIL
FLUNITRAZEPAM
(BZ)
BZ/BC
RECOGNITION
SITE
DMCM
(BC)
+
−
GABA
Picrotoxin
α
β
γ
β
α
Cl⁻

Figure 7: Hypothetical model for the GABA$_A$/benzodiazepine chloride ionophore receptor complex. Reprinted by permission of Elsevier Science Publishing Co., Inc. from Allosteric modulatory centers for transmitter amino acid receptors, by E. Costa, *Neuropsychopharmacology, Volume 2*, p. 170. Copyright 1989 by the American College of Neuropsychopharmacology.

In addition to the GABA and benzodiazepine binding sites described above, the GABA/benzodiazepine receptor complex also contains bindings sites for several other pharmacological agents: [1] picrotoxin site, recognizing convulsant agents like picrotoxin (Squires *et al.*, 1983) or t-butylbicyclophosphorothionate (Van Renterghem *et al.*, 1987) that block the GABA-activated channel; [2] depressant site, where central nervous system-depressant drugs like barbituates, at low concentrations, increase the open life-time of the Cl^- channel (Huang and Barker, 1980) and [3] site(s) that can bind the channel-permeating anions [but not other ions] (Squires, 1986). Each of these recognition sites can interact allosterically with one or more of the other sites (Squires, 1986), resulting in multiple modulatory inputs on the functioning of the GABA synapse.

F.3 *GABA$_B$ Receptors*

The term "GABA$_B$" site, first designated by Hill and Bowery (1981), described a receptor for GABA that was neither activated by traditional GABA$_A$ agonists like isoguvacine or piperidine-4-sulfonic acid nor blocked by the GABA$_A$ antagonist bicuculline (Curtis *et al.*, 1971). Evidence for GABA$_B$ receptors was initially noted in the mammalian peripheral nervous system where GABA was found to inhibit evoked neurotransmitter release from autonomic nerve terminals by a Cl^- ion-independent process (Bowery and Hudson, 1979; Bowery *et al.*, 1981). Evidence for the existence of GABA$_B$ receptors in the central nervous system soon followed based on a similar pharmacological profile for the inhibition of evoked neurotransmitter release from brain slices (Bowery *et al.*, 1980; Schlicker *et al.*, 1984) and the binding of [^{3}H]-GABA to rat brain synaptic membranes (Hill and Bowery, 1981). The most important compound used in the early characterization of GABA$_B$ receptors was baclofen β-(p-chlorophenyl)-GABA. Originally designed as a lipophilic derivative of GABA which was able to cross the blood-brain barrier (Bowery, 1982), baclofen was shown to be a stereoselective agonist for the $GABA_B$ receptor [(-)-baclofen which exhibits 100-fold greater activity than (+)-baclofen (Bowery, 1983)] and is inactive at the $GABA_A$/benzodiazepine receptor complex (Bowery, 1982).

Radiolabelled-ligand binding studies of $GABA_B$ receptors have been carried out with both [^{3}H]-GABA and [^{3}H]-baclofen (Bowery *et al.*, 1982). Analysis of these binding studies suggests that $GABA_B$ receptors may exist in several affinity states (Karbon *et al.*, 1983), with binding being dependent on the presence of divalent cations, most notably Ca^{2+} (Hill and Bowery, 1981). The high affinity $GABA_B$ binding site has a dissociation constant (Kd) in the 30-60 nM range (see Table 2), while the lower-affinity site has a Kd value between 100 and 200 nM (Enna and Karbon, 1986). There also appears to be a Na^+-dependent component to $GABA_B$ binding. Using [^{3}H]-baclofen and Krebs-Henseleit solution (which contains Na^+) or 50 mM Tris.HCl (containing 2.5 mM $CaCl_2$ and 143 mM NaCl) as incubation media, a very low affinity (ca.6000 nM) binding site has been observed in rat brain synaptic membranes (Bowery *et al.*, 1983a). According to these authors, this very low affinity site is not likely to be associated with the Na^+-dependent transporter since in the same study [^{3}H]-baclofen was not accumulated by brain slices under conditions in which [^{3}H]-GABA was rapidly taken up. It is not presently known whether these different receptor affinity states are due to distinct separate receptors or just different conformations of the same receptor (Enna and Karbon, 1986). The finding by Karbon *et al.* (1983) that lesions of the dorsal noradrenergic bundle cause a selective reduction in the number of lower-affinity sites, without altering the higher affinity sites, suggests that the two sites may be distinct entities.

The distribution of $GABA_B$ sites in mammalian brain has been determined both with radioligand binding assays and receptor autoradiography (Karbon *et al.*, 1983; Genhert *et al.*, 1985; Bowery *et al.*, 1987). As was the situation for the $GABA_A$/benzodiazepine receptor complex, a heterogeneous distribution of $GABA_B$ sites exists within the brain (see Table 1). The regions that exhibit the highest concentrations of $GABA_B$ sites are the cerebral cortex (especially the frontal cortex), hippocampus, thalamus, basal ganglia and cerebellum (Bowery *et al.*, 1987). Several of these brain regions contain high concentrations of both $GABA_A$ and $GABA_B$ receptors, but according to Bowery *et al.* (1989) there is no correlation between their distributions. This independence is supported by mapping studies of the cerebellum where $GABA_A$ sites predominate in the granule cell layer while $GABA_B$ sites are highly localized in the molecular cell layer (Palacios *et al.*, 1980; Wilkin *et al.*, 1981). In the spinal cord the highest concentrations of $GABA_B$ sites are in laminae II and III of the dorsal horns, while $GABA_A$ sites are quite uniformly distributed throughout the grey matter (Price *et al.*, 1984a).

Autoradiography has also been useful in the identification of $GABA_B$ sites at the cellular level. Results from selective lesioning studies also provide good evidence for the presence of $GABA_B$ receptors on both presynaptic nerve terminals and postsynaptic sites in many brain regions. In the interpeduncular nucleus at least 90% of $GABA_B$ sites appear to be on afferent terminals from the habenula (Price *et al.*, 1984b) and up to 50% of $GABA_B$ binding sites in the dorsal horn of the rat spinal cord are found on small diameter afferent fibers (Price *et al.*, 1987). A presynaptic location for $GABA_B$ sites is supported by lesioning experiments on the cerebral cortex and striatum. Decortication significantly reduced $GABA_B$ binding in the striatum, whereas kainic acid injected directly into the striatum produced no effect on the number of $GABA_B$ sites (Kilpatrick *et al.*, 1983). Presynaptic positioning has also been inferred by Ault and Nadler (1982) from their work on the rat hippocampal slice. They suggested that bicuculline-insensitive $GABA_B$ sites may be on glutamate and/or aspartate synaptic terminals, where they modulate the release of these acidic amino acid transmitters.

A postsynaptic location for $GABA_B$ receptors has been identified in the Purkinje cell dendrites of the cerebellum (Bowery *et al.*, 1983b) and in the CA1 pyramidal cells of the hippocampus (Dutar and Nicoll, 1988). In the study by Dutar and Nicoll, they found that the $GABA_B$-mediated postsynaptic hyperpolarization produced by GABA or baclofen could be blocked by the pretreatment with pertussis toxin, a chemical previously demonstrated to decrease the binding of [^{3}H]-GABA to $GABA_B$ receptors (Asano *et al.*, 1985). In addition, electrophysiological studies on cells in the cerebral cortex and thalamus have identified a slow inhibitory postsynaptic potential resulting from the activation of $GABA_B$ receptors. The hyperpolarization is due to an increase in K^+ conductance, and can be antagonized by the selective $GABA_B$ antagonist phaclofen (Soltesz *et al.*, 1988; Karlsson *et al.*, 1988).

As indicated above, $GABA_B$ receptor activation is not associated with a Cl^- ion channel. Instead, $GABA_B$ receptors appear to be linked to a variety of cellular compounds including the cations Ca^{2+} and K^+ as well as the second messenger cAMP (Dunlap, 1981; Wojcik and Neff, 1984; Deisz and Lux, 1985; Robertson and Rowland-Taylor, 1986; Nicoll and Dutar, 1989). The process(es) by which $GABA_B$ receptor activation alters these compounds is the subject of much intense research. Presently, there are four transduction systems through which $GABA_B$ receptors appear to operate: (1) inhibition of adenylate cyclase; (2) facilitation of transmitter-mediated activation of adenylate cyclase; (3) opening of K^+ channels; and (4) closing of Ca^{2+} channels.

In synaptosomal membranes from various rat brain regions, GABA and (-)-baclofen have been shown to inhibit adenylate cyclase activity with half maximal concentrations (EC_{50}) of 17 μM and 4 μM, respectively (Wojcik and Neff, 1984). This inhibition was not blocked by bicuculline nor facilitated by benzodiazepines, consistent with the activation of $GABA_B$ receptors (Wojcik *et al.*, 1989). Moreover, Wilkin *et al.*

(1981) have shown that a linear correlation exists between the maximal extent of $GABA_B$-mediated inhibition of adenylate cyclase and the binding of [^{3}H]-GABA to $GABA_B$ sites in several brain regions (Fig. 8). An inhibition of adenylate cyclase has also been observed in primary cultures of cerebellar granule cells and regional brain slice preparations both in the presence and absence of forskolin (Hill, 1985; Karbon and Enna, 1985; Xu and Wojcik, 1986), a direct activator of the catalytic subunit of adenylate cyclase (Seamon and Daly, 1981). $GABA_B$ receptors are thought to inhibit adenylate cyclase by coupling to an inhibitory guanine nucleotide (G_i, G_o) protein (Asano *et al.*, 1985; Xu and Wojcik, 1986). Two experimental findings consistent with this G-protein coupling are the guanine triphosphate (GTP) inhibition of ligand binding to $GABA_B$ binding sites (Hill *et al.*, 1984) and an attenuation of the inhibitory $GABA_B$ effect on adenylate cyclase by the pretreatment with islet activating protein [IAP] (Xu and Wojcik, 1986). IAP is a pertussis toxin that inactivates G_i and G_o proteins (Wojcik *et al.*, 1989).

The second effect on adenylate cyclase attributed to $GABA_B$ receptors is a facilitation of neurotransmitter-mediated activation of the enzyme adenylate cyclase. In brain slice preparations from every brain region except cerebellum, the $GABA_B$ agonists GABA and (-)-baclofen augment the cAMP accumulation that is stimulated by receptor activators of adenylate cyclase [e.g., isoproterenol, adenosine, vasoactive intestinal peptide and histamine] (Hill, 1985; Karbon and Enna, 1985; Watling and Bristow, 1986). The response is not inhibited by bicuculline (Wojcik *et al.*, 1989), indicating mediation through $GABA_B$ receptors. Three characteristics of this $GABA_B$ process are very puzzling. First, there is no $GABA_B$-mediated potentiation of cAMP production in the cerebellum (Karbon and Enna, 1985), despite the fact that the cerebellum contains one of the highest concentrations of $GABA_B$ receptors (Hill and Bowery, 1981). Second, the potentiating effects of $GABA_B$ agonists are observed in

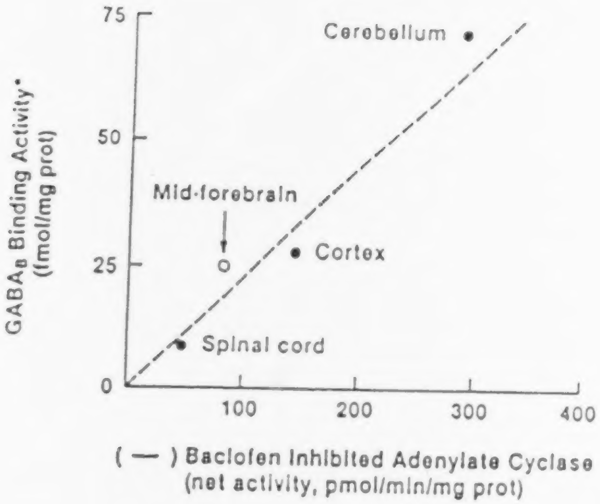


Figure 8: Linear correlation between $GABA_B$ recognition sites and $GABA_B$ receptor-mediated inhibition of adenylate cyclase activity in crude synaptosomal membranes prepared from various rat brain regions. Reprinted by permission of Raven Press from E.A. Barnard and E. Costa (eds.), *Allosteric Modulation of Amino Acid Receptors: Therapeutic Implications*, W.J. Wojcik, X. Paez and M. Ulivi, A transduction mechanism for $GABA_B$ receptors, 1989, p. 176.

brain slices but not in membrane preparations (Bowery *et al.*, 1989). Third, if adenylate cyclase in slices is activated directly by forskolin and not through a receptor-mediated mechanism, $GABA_B$ receptor activation results in an inhibition of adenylate cyclase (Wojcik *et al.*, 1989). Enna and Karbon (1987) proposed that this $GABA_B$ facilitation is mediated through activation of phospholipase A_2, which then leads to increased cAMP accumulation. This conclusion was based mainly on the findings that quinacrine and corticosteriods (both thought to inhibit phospholipase A_2) attenuated the $GABA_B$ potentiation of β-adrenoceptor-stimulated cAMP production. Much work remains to be done in order to identify whether $GABA_B$ receptors are directly coupled to phospholipase A_2 and which metabolic events link the phospholipase A_2 activation to the cAMP accumulation (Wojcik *et al.*, 1989). In this regard, it has been demonstrated that $GABA_B$ receptors activate phospholipase A_2 and release arachidonic acid from plasma membranes (Duman *et al.*, 1986).

A transduction mechanism through membrane K^+ channels has also been suggested for $GABA_B$ receptors. Intracellular recording from hippocampal and substantia nigra cells shows that baclofen and GABA produce a bicuculline-insensitive hyperpolarization via the opening of K^+ channels (Newberry and Nicoll, 1984; Pinnock, 1984; Gahwiler and Brown, 1985; Inoue *et al.*, 1985; Ogata *et al.*, 1987). The involvement of K^+ ions in this response is supported by findings of Newberry and Nicoll (1985) who found the hyperpolarization to be associated with an increase in membrane conductance and a reversal potential of about -90 mV (consistent with the K^+ equilibrium potential). In addition, increasing extracellular K^+ concentrations caused a depolarizing shift in the reversal potential, while manipulation of Cl^- ion concentrations had little effect on the hyperpolarization (Nicoll and Dutar, 1989). The mechanism by which $GABA_B$ receptors open K^+ channels appears to be indirect, with coupling to a

G-protein likely. Support for such an association includes blocking of the actions of baclofen on K^+ conductance by pertussis toxin and potentiation of its effects by the GTP analogue GTPγS (Andrade *et al.*, 1986).

The fourth and, at present, final transduction process linked to $GABA_B$ receptors is a closure of Ca^{2+} channels. Experiments utilizing primary cultures of chick and rat dorsal root ganglion cells have demonstrated that $GABA_B$ receptor activation reduces Ca^{2+} influx through long-lasting voltage-dependent Ca^{2+} channels (Dunlop, 1981; Holz *et al.*, 1986; Dolphin and Scott, 1986). In agreement with the previous transduction mechanisms, the closure of these Ca^{2+} channels is also mediated through a pertussis toxin-sensitive G-protein (Holz *et al.*, 1986; Ohmori *et al.*, 1990). With respect to the functional significance of this transduction mechanism, presynaptic location for $GABA_B$ receptors coupled to Ca^{2+} channels would provide a direct method for regulating Ca^{2+} fluxes thought to be intimately involved in the control of neurotransmitter release. $GABA_B$ receptor activation has been shown to inhibit the release of several brain neurotransmitters, including 5-HT (Bowery *et al.*, 1980; Gray and Green, 1987), glutamate and aspartate (Potashner, 1980; Kato *et al.*, 1982), noradrenaline and dopamine (Bowery *et al.*, 1980) and GABA [through a $GABA_B$-like autoreceptor] (Bonanno *et al.*, 1989; Raiteri *et al.*, 1989).

G. RECEPTOR BINDING ASSAYS

G.1 *General Principles*

The aim of receptor binding analysis is to label and quantitatively assay the receptors (binding sites) under investigation so that information about the receptors' functioning and potential for pharmacological manipulation can be determined. A question fundamental to this analysis is to what extent is the binding site equivalent to a functional receptor (Burt, 1980). The key property of a binding site which distinguishes it as a receptor is its association with a function (i.e., coupled to a response). Information about binding site function is not, however, available from a traditional receptor binding assay. For this reason, binding data from an experiment should always be correlated with measurements of biochemical, electrophysiological or behavioural responses, preferably in the same tissue (Burt, 1980).

In the identification and characterization of a radioligand binding assay, the binding of the radioligand to the assay preparation must meet certain basic criteria in order to be recognized as biologically meaningful binding to a specific receptor: (1) saturation and reversibility, and (2) physiological and pharmacological specificity (Hrdina, 1986).

The minimal requirement for binding to be of biological interest is that it be saturable. There is only a finite number of specific receptor sites per unit of tissue, and as the concentration of radioligand increases these sites become fully occupied. The binding of the radioligand to "nonspecific" sites, such as filters and glassware, is not saturable within a reasonable range of radioligand concentrations. Reversibility of binding is most easily illustrated through an examination of how specific and nonspecific binding components are determined. In a typical saturation binding assay, a fixed amount of tissue is incubated with increasing concentrations of the radioligand in the presence (nonspecific binding) or absence (total binding) of an excess amount of nonradiolabelled molecules of a compound which compete for the receptor. Specific binding is then defined as the difference in radioactivity between total and nonspecific binding. Nonspecific binding can be determined by the addition of unlabelled drug because through competition at the specific receptor, the reversibly bound radioligand is displaced by the unlabelled compound. If the radioligand had been irreversibly bound, no competitive displacement of the ligand could have occurred. By default the radioactive ligand not displaced must be bound to nonspecific sites. A binding curve outlining the relationship between these 3 components is shown in Fig. 9.

Physiological or pharmacological specificity is the second basic requirement of binding site/receptor characterization. The most important feature of pharmacological specificity is a high degree of correlation between the potencies of unlabelled drugs to displace the radioligand from the specific binding site and their potencies in producing a biological response (Burt, 1978). A strong correlation should also exist between the affinity of a drug for the specific binding site *in vitro* and its pharmacological potency *in vivo* (Hrdina, 1986). As pointed out by Burt (1978), when identifying binding site specificity it is not sufficient just to determine that drugs of different structures do not compete for binding. Such selectivity would in fact be expected of any relatively high-affinity binding site due to the multiple interactions which contribute to the tight binding. For receptor identification, the correlation with biological effects is an essential consideration. An additional indication of pharmacological specificity that has been utilized is stereospecificity of binding (Hrdina, 1986).

G.2 *Methodology*

Radiolabelled ligands used in receptor binding assays can be either agonists or antagonists for a given receptor (binding) site. Important characteristics of good radioligands include purity, stability, biological activity and sufficiently high specific activity (Bennett, 1978). Purity and stability are important for proper interpretation of binding results due to the possibility that impurities and decomposed compounds might bind with less specificity than the native compound (Hrdina, 1986). Biological activity of a ligand is important because during the radiolabelling process the ligand's structural and pharmacological features may have been modified. This possibility is greatest with

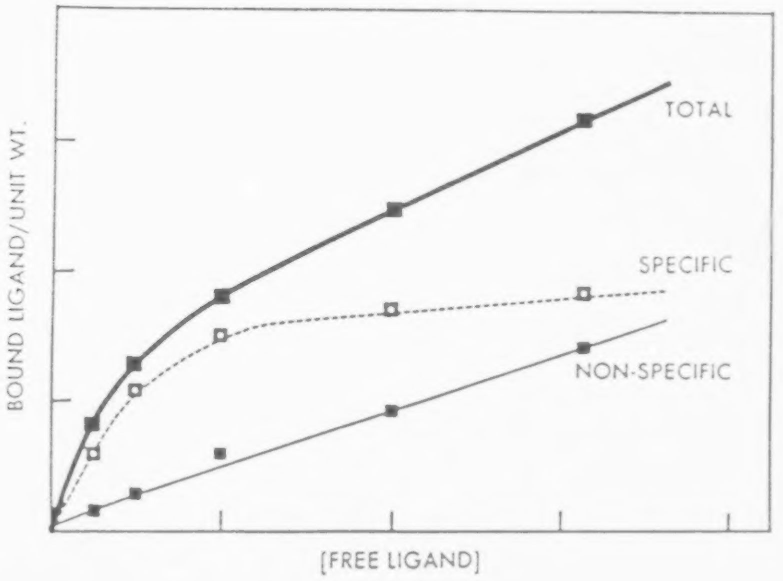


Figure 9: Typical binding curves generated from a direct binding assay. Reprinted with permission by Humana Press from *Neuromethods, Volume 4, Receptor Binding*, A.A. Boulton, G.B. Baker and P. Hrdina (eds.), 1986, p. 8.

iodinated compounds and least likely with tritiated compounds (Bennett, 1978). High specific activity (defined as the amount of radioactivity present in a given weight or molar unit of a compound) is important to allow for accurate measurements of radioactivity at low radioligand concentrations (Hrdina, 1986).

As outlined earlier under general principles, a typical saturation binding assay involves incubation of a known concentration of tissue and increasing concentrations of radioligand with (nonspecific binding) and without (total binding) an excess of unlabelled displacer. In the ideal case, this incubation would occur under conditions of temperature, pH and incubation medium that resemble the *in situ* situation (Hrdina, 1986). Realistically, optimal binding conditions are determined through trial and error and typically are those conditions that yield the greatest amount of specific binding.

Measurement of the amount of radioligand bound to the tissue under study requires separation of bound from unbound (free) radioligand. The choice of a separation technique depends on whether the receptor under study is in particulate or soluble form (Bennett, 1978). Since all of the binding assays utilized in this thesis are of the particulate form, only this preparation will be discussed. For particulate binding the choice of separation technique is usually between some form of centrifugation or filtration. The major constraint that determines which of these techniques is used is the rate of dissociation of the receptor-ligand complex (see below for details). For example, with receptor-ligand complexes with rapid dissociation kinetics (e.g. $GABA_B$ binding), centrifugation is often used. Conversely, for receptor-ligand complexes with slower dissociation (e.g. β-adrenergic binding) filtration is frequently the method of choice (Bennett, 1978).

Receptor binding studies usually follow kinetics that are very similar to those described for classic enzyme-substrate interactions and follow the law of mass action.

A reversible receptor-ligand interaction where R = concentration of unoccupied receptor sites, L = concentration of unbound ligand and RL = concentration of receptor-ligand complex, and K_1 and K_{-1} represent the rate of the forward and reverse reactions respectively, can be described as in equation (1)

Equation (1)

$$[R]+[L] \overset{K_1}{\underset{K_{-1}}{\Leftrightarrow}} [RL]$$

At equilibrium $K_1 [R][L] = K_{-1} [RL]$ and the equilibrium dissociation constant (K_d) can be defined as in equation (2).

Equation (2)

$$K_d = \frac{K_{-1}}{K_1} = \frac{[R][L]}{[RL]}$$

A second value of interest in binding studies is the maximum number of specific binding sites (Bmax). Both Bmax and K_d can be estimated from a Scatchard plot of the saturation binding data. The Scatchard equation (Scatchard, 1949) is shown below [Equation (3)], and represented graphically in Figure 10. K_d is equal to the negative reciprocal of the slope of the line and the Bmax is equal to the x-axis intercept (i.e., where B/F = 0).

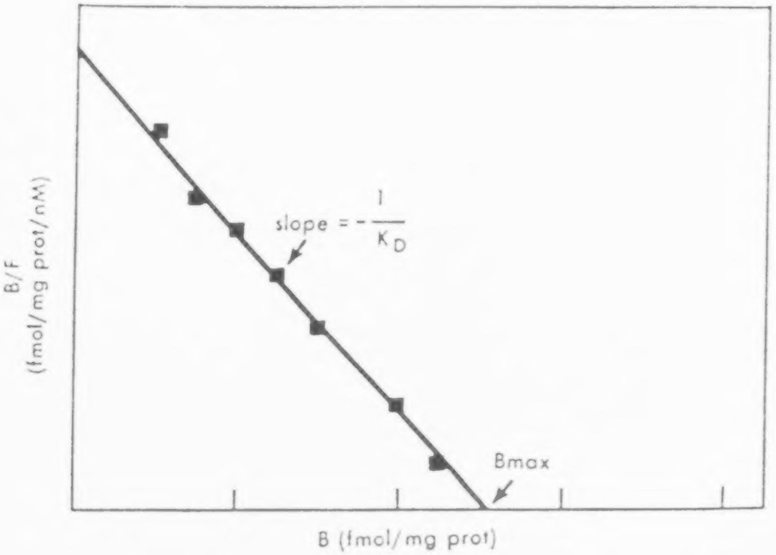


Figure 10: A typical Scatchard plot of binding data. Reprinted with permission by Humana Press from *Neuromethods, Volume 4, Receptor Binding*, A.A. Boulton, G.B. Baker and P. Hrdina (eds.), 1986, p. 10.

Equation (3)

$$\frac{B}{F} = \frac{Bmax - B}{K_d}$$

B = bound ligand; F = unbound (free) ligand

H. OBJECTIVES OF THIS STUDY

An overview of the experimental evidence for antidepressant drug-induced changes in GABAergic activity given in Sections B.2 and D.5 of the introduction reveals a considerable degree of controversy. The present study involved both a behavioral and neurochemical assessment of brain GABA changes, and it was hoped that this combined approach would provide a more comprehensive picture of the involvement of GABA in the mechanism(s) of action of antidepressant drugs.

The two main objectives of this investigation were as follows: (1) to assess changes in $GABA_A$, $GABA_B$ and β-adrenoceptor number and function following chronic antidepressant drug treatment; and (2) to assess the influence of chronic antidepressant drug treatment on 3 indices of GABA metabolism - GABA levels and GAD and GABA-T enzyme activities.

MATERIALS AND METHODS

A. CHEMICALS

Table 3: Chemicals used in the studies described in this thesis.

Chemicals	Suppliers
acetic acid - glacial	BDH Chemicals (Toronto, ON)
acetic anhydride	Caledon Laboratories (Georgetown, ON)
acetonitrile, HPLC grade distilled in glass	BDH Chemicals
alprenolol HCl	Sigma
γ-aminobutyric acid	Aldrich (Milwaukee, WI)
aminobutyric acid, γ-[2,3-^{3}H(N)]-	Dupont, NEN Products (Boston, MA)
2-amino-ethylisothio-uronium bromide	Sigma
2-amino-2-hydroxymethylpropane-1,3-diol	Fisher Scientific (Edmonton, AB)
ascorbic acid	Fisher Scientific
(±)-baclofen	Ciba-Geigy (Summit, NJ)
(+)-baclofen	Ciba-Geigy

bovine serum albumin	Sigma
calcium chloride	Fisher Scientific
chloroform, reagent grade	Fisher Scientific
citric acid	Anachemia Ltd. (Edmonton, AB)
cupric sulfate	Fisher Scientific
D,L-isoleucine	Sigma
D,L-norleucine	Aldrich
D,L-valine	Nutritional Biochemical Corp. (Cleveland, OH)
deoxycholate	Fisher Scientific
desmethylimipramine HCl	Sigma
dicyclohexylcarbodiimide	Aldrich
diethyl ether	BDH Chemicals
dihydroalprenolol,levo[ring,propyl-^{3}H]	Dupont, NEN Products
dithiothreitol	Sigma
ethyl acetate	BDH Chemicals
ethylenediamine tetraacetate, disodium salt	Fisher Scientific
folin - phenol reagent	Sigma
glutamic acid L-[1-^{14}C]-	Amersham Canada Ltd. (Oakville, ON)

glutathione	Sigma
glycerol	Sigma
hydrochloric acid, 37-38%	Fisher Scientific
hydroxytryptamine binoxalate 5-[2-^{14}C]	Dupont, NEN Products
5-hydroxytryptamine creatine sulfate	Sigma
imipramine HCl	Sigma
iso-pentane	BDH Chemicals
isobutyl chloroformate	Aldrich
isoguvacine HCL	Research Biochemicals Inc. (Natick, MA)
α-ketoglutarate	Sigma
L-alanine	Aldrich
L-glycine	Sigma
L-leucine	Sigma
maprotiline	Ciba-Geigy
muscimol	Sigma
muscimol, [methylene-^{3}H(N)]-	Dupont, NEN Products
nicotinamide adenosine dinucleotide	Sigma
pentafluorophenol	Aldrich
perchloric acid, 60%	Fisher Scientific

phenelzine sulfate	Sigma (St. Louis, MO)
β-phenylethylamine HCl	Sigma
β-phenylethylamine HCl 2-[ethyl-1-^{14}C]	Dupont, NEN Products
phosphoric acid, 85%	Fisher Scientific
potassium biphosphate	J.T. Baker Chemicals Co. (Phillipsberg, NJ)
potassium carbonate anhydrous	Fisher Scientific
potassium chloride	Fisher Scientific
potassium dihydrogen orthophosphate	Fisher Scientific
potassium hydrogen orthophosphate triphosphate	Fisher Scientific
progabide	LERS - Synthelabo, France
protosol	Dupont, NEN Products
pyridoxal phosphate	Sigma
salbutamol hemisulfate	Sigma
scintillation fluid (Ready Safe)	Beckman Instruments Inc. (Edmonton, AB)
sodium bicarbonate	Fisher Scientific
sodium bicarbonate	Fisher Scientific
sodium carbonate anhydrous	Fisher Scientific

sodium chloride	Fisher Scientific
sodium hydroxide	Fisher Scientific
sodium phosphate, dibasic, anhydrous	Fisher Scientific
sodium phosphate, monobasic	Fisher Scientific
sodium potassium tartrate	Allen & Hanbury's (Toronto, ON)
sucrose	Fisher Scientific
toluene, glass-distilled	BDH Chemicals
toluene, reagent grade	BDH Chemicals
(±)-tranylcypromine HCl	Sigma
tri-n-octylamine	Sigma
TRIS (hydroxymethyl) aminomethane	Fisher Scientific
Triton X-100	Terochem Lab. Ltd. (Edmonton, AB)

B. INSTRUMENTATION AND APPARATUS

B.1 *Locomotor Activity Monitoring System*

The activity monitoring system (Acadia Insts. Ltd., Saskatoon, Saskatchewan, Canada) consisted of six 17" x 17" x 12" plexiglass test cages each placed in a 12 x 12 beam infra-red grid system (Acadia Infra-red Grid Model 17-12 with vertical sensors). The test cages' sensors were interfaced with a microcomputer system (Acadia 6502 Data Gatherer) for data-logging and temporal analysis of activity counts.

B.2 *Filtration*

A Brandel Cell Harvester equipped with Whatman GF/C filters was used for the filtration step in [^{3}H]-dihydroalprenolol (DHA) and [^{3}H]-muscimol receptor binding assays.

B.3 *Centrifuges*

A Sorvall GLC-2B or Sorvall GLC-1 General Laboratory Centrifuge (Dupont Instruments) was used for low-speed, small volume centrifugations. Higher speed and/or larger volume centrifugations were carried out in a Damon-IEC B-20 refrigerated high-speed centrifuge or a Beckman L755 vacuum refrigerated ultracentrifuge.

B.4 *Gas Chromatography*

For tricyclic antidepressant level determinations a Hewlett Packard (HP) Model 5890 gas chromatograph equipped with a fused silica column and a nitrogen phosphorus detector linked to an HP 3392A integrator was used. The carrier gas was pure helium at a flow rate of 1-2 ml/min. The detector was purged with pure hydrogen (3.5 ml/min) mixed with dry air at 80 ml/min. The injection port temperature was 200°C and the detector temperature was 325°C.

For amino acid level determinations a HP 5890 gas chromatograph equipped with a fused silica column, an electron-capture detector with a radioactive source of 15 mCI Nickel-63, an HP 7673A automatic sampler and an HP 3392A integrator was used. The carrier gas, helium, was set at a flow rate of 2 ml/min. Argon-methane (95%-5%), flow rate 35 ml/min, was the make-up gas used in the detector. The injection port temperature was 200°C and the detector temperature was 325°C.

B.5 *Liquid Scintillation Spectrometry*

A Beckman LS 7500 liquid scintillation spectrometer coupled to a Datamex 43 printer was used for counting radioactivity in all *ex vivo* receptor binding and MAO inhibition studies.

B.6 *Ultraviolet Spectrophotometer*

A Pye Unicam SP 1700 ultraviolet spectrophotometer was used for determination of protein concentrations in receptor binding homogenates.

B.7 *Tissue Homogenizer*

A combination of a TRI-R S63C variable speed laboratory motor with a Teflon glass pestle and a glass grinding tube was used for homogenizing tissue samples.

B.8 *Shaker-Mixer*

Two types of vortex-shakers were used: Ika-Vibrax VXR2 Shaker (Janke and Kunkel Instruments) and a thermolyne Maxi Mix vortex mixer (Sybron/Thermolyne Instruments).

B.9 *Weighing Balances*

A Mettler AE 160 electronic balance was used for weighing chemicals and biological samples.

B.10 *Glass Cleaning*

All glassware was rinsed with tap water and washed out with biodegradable Sparkleen (Fisher Scientific Co.) solution. Further washing was accomplished with a dishwasher (Miele Electronic 6715). For test tubes, an additional cleaning step was added; test tubes were sonicated (Ultra-sonic cleaner, Mettler Electronics) in a solution of Decon 75 concentrate (BDH Chemicals) before the dishwasher wash. All glassware was then air-dried in a mechanical convection oven Model 28, Precision Scientific Group.

C. ANIMALS

Male Sprague-Dawley rats weighing 275-325 g were purchased from Bio-Science Animal Services, Ellerslie, Alberta. The animals were group housed (2 per cage) under a 12 h light/dark cycle at a temperature of 20 ± 1°C. Food and water were freely available. The animal feed (Lab-Blox feed, Wayne Feed Division, Continental Grain Co., Chicago, USA) composition was 4.0% crude fat (min), 4.5% crude fibre (max) and 24% crude protein (min).

All animal experimentation described in this thesis was approved by the University of Alberta Animal Care Committee in accord with the Canadian Council on Animal Care (CCAC) guidelines.

C.1 *Surgery and Drug Administration*

Animals were randomly allocated to drug or vehicle treatment conditions. Each animal was deeply anesthetized with a mixture of ether and air, and an osmotic mini-pump (Alzet 2ML4, Alza Corp., Palo Alto, CA) was implanted sc in the dorsal thoracic region. Each pump was filled with a drug solution individually adjusted in concentration

(Greenshaw, 1986) or the distilled water vehicle according to each animals' group allocation to provide constant infusion for a total of 28 days. This 28 d test period was chosen because it allowed for a wash-out period of the receptor agonists [administered on days 21 and 22 of chronic antidepressant drug administration (see below)] prior to the assessment of receptor binding changes after 28 d of antidepressant treatment. Drug treatments were as follows: PLZ sulfate 5, 10 mg/kg/d; (±)-TCP hydrochloride 1 mg/kg/d; IMI hydrochloride 30 mg/kg/d; DMI hydrochloride 5, 10 mg/kg/d. The incisions were sutured and, after recovery, the animals were placed in normal housing conditions.

On days 21 and 22 of chronic drug administration pharmacological challenges were conducted as *in vivo* behavioural tests of β-adrenergic and $GABA_B$ receptor function. The functional tests are described in Section D.

C.2 *Sample Collection and Storage*

Following 28 d of drug administration the animals were killed by rapid decapitation and their brains removed and dissected over ice. Frontal cortex, remainder of the cerebral cortex and rest of brain were stored at -80°C until neurochemical analysis.

D. FUNCTIONAL ANALYSIS

In vivo assessments of receptor sensitivity to pharmacological challenge were conducted on days 21 and 22 of chronic drug administration. The effects on spontaneous locomotor activity of receptor agonist [salbutamol (3 mg/kg); progabide (50 mg/kg); or (±)-baclofen (5 mg/kg)] and of the 0.9% saline vehicle were assessed in a counterbalanced manner. A randomly selected half of each treatment group received an injection containing one of the receptor agonists on day 21 and saline on day 22. This order of drug/saline was reversed for the remaining animals to control for possible order effects. All injections were i.p. 15 min prior to behavioural testing in a volume of 1 ml/kg [salbutamol, (±)-baclofen] or 10 ml/kg (progabide). The testing procedure involved placement of the animals individually into computer-controlled infra-red activity measurement systems for 30 min, under conditions of reduced illumination in a quiet test environment.

Prior to the performance of the functional tests, dose-responses for each GABA receptor agonist were measured with separate groups of naive rats in order to determine the most appropriate dose for the pharmacological challenges. In addition, (±)-baclofen and (+)-baclofen were tested in separate groups of animals to assess the stereoselectivity of the behavioural response to this ligand. For these estimates, animals were injected with a single dose of one of the agonists or the 0.9% saline vehicle. The injections were i.p., 15 min prior to placement of the animals in the activity measurement system (described in Section B.1) for 30 min. The salbutamol dose was chosen on the basis of previous pilot studies and a consideration of the relevant literature (Przegalinski *et al.*, 1983, 1984).

E. RECEPTOR BINDING ANALYSIS

E.1 *[^{3}H]-Dihydroalprenolol (DHA) Binding*

The procedure for measuring [^{3}H]-DHA binding to total cortical β-adrenoceptors was adapted from that of Bylund and Snyder (1976). Cerebral cortex tissue from a single animal was used for each saturation binding curve. Cerebral cortices were homogenized in 10 volumes of ice-cold 50 mM Tris.HCl buffer (pH=7.4). The homogenate was decanted into centrifuge tubes and buffer was added to yield a total dilution of 100 volumes. This homogenate was centrifuged at 40,000 x g at 4°C for 10 min. The supernatant was discarded and the pellet resuspended in 10 volumes of buffer, subsequently made up to a 100 volume dilution. The resultant suspension was recentrifuged and washed once again as above. After the second centrifugation, the final pellet was resuspended in 10 volumes of buffer. Aliquots (100 μl) of this final suspension containing approximately 0.5 to 0.6 mg protein per ml were then incubated at 23°C in the presence of 0.25 to 5.0 nM of [^{3}H]-DHA.

Saturation analyses were performed in triplicate in a final volume of 1 ml. Binding was terminated by rapid filtration. Filters were washed 3 times with approximately 4 ml of ice-cold buffer. Non-specific binding was estimated from parallel assay tubes containing 10 μM alprenolol for each concentration of [^{3}H]-DHA. Following filtration the filters were removed, dried and placed into scintillation vials containing 5 ml of scintillation fluid (Ready Safe, Beckman). After 12 h the vials were vortexed and counted for 10 min in a liquid scintillation spectrometer. Specific binding, defined as the difference between total and non-specific binding, was 85%. An outline of [^{3}H]-DHA binding parameters is shown in Table 4. For all receptor binding studies, Scatchard and Ligand analysis was performed using the computer programs of McPherson (1985).

E.2 *[^{3}H]-GABA Binding*

The measurement of the binding of [^{3}H]-GABA to GABA$_B$ binding sites was essentially as described by Hill and Bowery (1981). Frontal cortices from 2-3 animals were pooled for each saturation binding curve. The cortices were homogenized in 10 volumes of ice-cold 0.32 M sucrose. A portion (300μl) of the homogenate was removed for use in the measurement of GAD and GABA-T activities and GABA levels. The rest of the homogenate was centrifuged at 1,000 x g for 10 min and the supernatant collected and recentrifuged at 20,000 x g for 20 min at 4°C. The pellet obtained from

$[^3H]$-DHA BINDING

Tubes	*Buffer (μl)*	*Alprenolol (μl)*	*$[^3H]$-DHA (μl)*	*Tissue (μl)*	*nM*
1-3/4-6	875/775	0/100	25	100	0.25
7-9/10-12	850/750	0/100	50	100	0.5
13-15/16-18	825/725	0/100	75	100	0.75
19-21/22-24	800/700	0/100	100	100	1.0
25-27/28-30	700/600	0/100	200	100	2.0
31-33/34-36	600/500	0.100	300	100	3.0
37-39/40-42	400/300	0/100	500	100	5.0

Table 4: Assay conditions used in the radioligand determination of $[^3H]$-DHA binding to β-adrenergic binding sites.

this second centrifugation was resuspended in distilled water (15 volumes) and centrifuged at 8,000 x g for 20 min at 4°C. The supernatant together with the buffy layer on the pellet was then centrifuged at 50,000 x g for 20 min at 4°C. The pellet was resuspended in distilled water (15 volumes) and again centrifuged at 50,000 x g for 20 min, 4°C. The final pellet was stored at -20°C for at least 24 h. On the day of the binding assay, the frozen pellet was thawed and resuspended in 15 volumes of ice-cold 50 mM Tris.HCl buffer (pH=7.4), containing 2.5 mM $CaCl_2$. Additional buffer was then added to yield a total of 100 volumes and this suspension was incubated at room temperature (23°C) for 45 min. Following the incubation, the suspension was centrifuged at 16,000 x g for 10 min at 4°C. The resultant pellet was resuspended (15 volumes buffer), subsequently made up to 100 volumes, incubated at 23°C for 15 min and centrifuged again at 16,000 x g for 10 min at 4°C. This final wash was repeated once. The final membrane suspension contained approximately 0.3 to 0.5 mg protein per ml.

For the binding assay, aliquots (500 μl) of the final membrane suspension were incubated for 10 min at 23°C in tubes containing isoguvacine (40 μM), $[^3H]$-GABA (30 Ci/mmol) (1 nM) and one of 7 concentrations of unlabelled GABA (5-160 nM) for a cold saturation curve. Buffer was added to a final volume of 1 ml. Non-specific binding was defined using (±)-baclofen (100 μM) and represented approximately 60% of total binding. Non-specific binding was determined at 1 nM $[^3H]$-GABA and was held constant at all unlabelled GABA concentrations. The incubation was terminated by centrifugation (48,000 x g, 20 min). The resultant pellet was washed briefly and superficially with 4 ml of ice-cold distilled water, followed by removal of the supernatant by aspiration. The pellet was dissolved in Protosol (Dupont, NEN Products) (300 μl) overnight before the addition of scintillation fluid (3 ml). After 12 h the samples were counted for 10 min (counting efficiency 43%) in a liquid scintillation spectrometer. An outline of $[^3H]$-GABA binding parameters is shown in Table 5.

[^{3}H]-GABA (1 nM) BINDING

Tubes	*Cold Sol'n (conc.)*	*Cold Sol'n (μl)*	*Buffer (μl)*	*Isoguvacine (μl)*	*[^{3}H]-GABA (μl)*	*Tissue (μl)*
1-3	(±)-baclofen: 100 μM (NSB)	100	350	40	10	500
4-6	GABA:5 nM	100	350	40	10	500
7-9	GABA:10 nM	100	350	40	10	500
10-12	GABA:20 nM	100	350	40	10	500
13-15	GABA:40 nM	100	350	40	10	500
16-18	GABA:80 nM	100	350	40	10	500
19-21	GABA:160 nM	100	350	40	10	500

Table 5: Assay conditions used in the radioligand determination of [^{3}H]-GABA binding to $GABA_B$ binding sites.

E.3 *[^{3}H]-Muscimol Binding*

[^{3}H]-Muscimol was used as radiolabel to determine the number and affinity of GABA$_A$ binding sites. Cerebral cortex tissue from a single animal was used for each saturation binding curve. Membrane preparation was identical to that described for [^{3}H]-GABA binding up to and including the formation of the pellet stored at -20°C for at least 24 h. On the day of the binding assay, the pellet was thawed, resuspended in 10 volumes of 50 mM Tris.citrate buffer (pH=7.1), containing 50 nM NaCl and centrifuged at 48,000 x g for 20 min at 4°C. The resultant pellet was resuspended in 40 volumes buffer, and incubated at 37°C for 30 min. Following the incubation period, additional buffer was added to yield a total of 100 volumes, and the suspension centrifuged at 25,000 x g for 20 min at 4°C. The resultant pellet was resuspended in 20 volumes buffer to give a final membrane suspension containing approximately 0.2 to 0.3 mg protein per ml.

For the binding assay, aliquots (200 μl) of the final membrane suspension were incubated for 60 min at 0°C in tubes containing [^{3}H]-muscimol (20 Ci/mmol) (5 nM) and one of 5 concentrations of unlabelled muscimol (2-32 nM). Buffer was added to a final volume of 1 ml. Non-specific binding was defined using unlabelled GABA (1 mM) and represented approximately 10% of total binding. Non-specific binding was determined at 5 nM [^{3}H]-muscimol and was held constant at all unlabelled muscimol concentrations. The incubation was terminated by rapid filtration. Filters were washed 3 times with approximately 4 ml ice-cold buffer per wash. Following filtration, the filters were placed in scintillation vials containing 5 ml scintillation fluid and allowed to sit for 12 h before counting in a liquid scintillation spectrometer. An outline of [^{3}H]-muscimol binding parameters is shown in Table 6.

[^{3}H]-MUSCIMOL (5 nM) BINDING

Tubes	*Cold Sol'n (conc.)*	*Cold Sol'n (μl)*	*Buffer (μl)*	*[^{3}H]-Muscimol (μl)*	*Tissue (μl)*
1-3	GABA 1 mM (NSB)	100	600	100	200
4-6	Muscimol:2 nM	100	600	100	200
7-9	Muscimol:4 nM	100	600	100	200
10-12	Muscimol:8 nM	100	600	100	200
13-15	Muscimol:16 nM	100	600	100	200
16-18	Muscimol:32 nM	100	600	100	200

Table 6: Assay conditions used in the radioligand determination of [^{3}H]-muscimol binding to $GABA_A$ binding sites.

F. ANALYSIS OF GABA AND OTHER ALIPHATIC AMINO ACIDS

A gas chromatographic (GC) assay developed by Wong *et al.* (1990a) was used for the simultaneous analysis of GABA, alanine, valine, leucine and isoleucine in rat frontal cortex. A 50 µl aliquot of homogenate from Section E.2 was vortexed and centrifuged at 1,000 x g for 2 min with 10% 1M perchloric acid (which contained 10 mg % disodium EDTA and 0.05 mM ascorbic acid). A 10µl aliquot of the clear supernatant was then used for the analysis. Norleucine (0.25 µg) was added as an internal standard to the supernatant; this was followed by the addition of 1 ml of 2.5% w/v potassium carbonate solution. One ml of an isobutyl chloroformate solution (5 µl isobutyl chloroformate in 1 ml of acetonitrile:toluene, 1:9 v/v) was added to the mixture. These solutions were vortexed for 15 min at room temperature. After a brief centrifugation, the top (organic) layer was aspirated and discarded. To the bottom (aqueous) phase was added 1.5 ml 2M sodium phosphate buffer, pH=5.3. This was followed by the sequential addition of 2.5 ml chloroform, 200 µl dicyclohexylcarbodiimide solution (5 µl in 1 ml chloroform) and 200 µl pentafluorophenol solution (5 µl in 1 ml chloroform). These solutions were vortexed for 15 min at room temperature. After a brief centrifugation, the top (aqueous) layer was aspirated and discarded. The bottom chloroform layer was then evaporated (at 60°C) to dryness under a stream of nitrogen. The residue was reconstituted in 300 µl toluene, which was then briefly washed with 1 ml distilled water. An aliquot of this toluene layer was used for GC analysis. Chromatographic separation was accomplished using the following automatic temperature program: initial temperature 100°C for 0.5 min, increasing to 200°C at a rate of 25°C/min; after remaining at 200°C for 0.5 min, the temperature increased at a rate of 3°C/min to a final temperature of 230°C. The chromatographic column used was a fused silica capillary column, cross-linked 5% phenylmethylsilicone phase, 0.31 mm I.D. x 25 m, 1.03 μm film thickness (Hewlett Packard, Palo Alto, CA, U.S.A.).

For all GC analyses described in this thesis, a standard (calibration) curve was prepared with each assay run to permit analysis of the quantity of the drug or neurochemical of interest in the brain homogenate supernatants. The curve was constructed by adding known, varying amounts of authentic standard and a fixed amount (same amount as added to the tissue supernatants) of internal standard to a series of tubes and running these tubes in parallel with the sample tubes.

G. DETERMINATION OF GABA-TRANSAMINASE ACTIVITY

GABA-transaminase (GABA-T) activity was measured with a modification of the procedure of Sterri and Fonnum (1978). Composition of the incubation medium was as follows: 1 μl [^{3}H]-GABA (30 Ci/mmol), 7.5 μl 100 mM GABA, 15 μl 50 mM α-ketoglutarate, 15 μl 10 mM nicotinamide adenosine dinucleotide, 15 μl 10 mM 2-aminoethylisothio-uronium bromide, 60 μl distilled water and 37.5 μl 50 mM Tris.HCl buffer (pH=7.9). To 1.5 ml microfuge tubes placed on ice, 5 μl of tissue homogenate from Section E.2 (with 1 μM pyridoxal phosphate added) [5 μl distilled water to blanks] and 20 μl incubation medium were added. The tubes were incubated at 37°C for 30 min, before the addition of 100 μl tri-n-octylamine. The mixture was vortexed briefly, followed by centrifugation of 1,000 x g for 2 min. An aliquot (35 μl) of the top layer was removed and added to a counting vial containing 4 ml of scintillation fluid. Radioactivity was measured in a liquid scintillation spectrometer after allowing the samples to sit overnight.

H. DETERMINATION OF GLUTAMIC ACID DECARBOXYLASE ACTIVITY

Glutamic acid decarboxylase activity was measured using a modification of the procedure of Albers and Brady (1959). Incubation medium contained the following: 20μl [^{14}C]-glutamic acid (59 mCi/mmol), 10μl 1M potassium phosphate buffer pH = 6.5, 5 μl 10 mM dithiothrietol, 5 μl 500 mM sodium glutamate and 60 μl distilled water. To glass tubes (cut to 2.5 cm) on ice were added 10 μl incubation medium and 5 μl brain homogenate from Section E.2 (with 1 μM pyridoxal phosphate added) (5 μl distilled water to blanks) were added. The glass tubes were connected through 7 cm polyethylene tubing to a second set of tubes containing 2.5 cm Whatman no. 1 filter papers and 150 μl Protosol. The connected tubes were incubated at 37°C for 30 min. The reaction was terminated by the addition of 50μl 6N sulfuric acid, and the tubes left to incubate another 40 min. Following the 40 min incubation, the glass tubes containing the filter papers were placed into scintillation vials containing 9 ml of scintillation fluid and 3 drops of glacial acidic acid. Radioactivity was counted in a liquid scintillation spectrometer.

I. DETERMINATION OF MONOAMINE OXIDASE ACTIVITY

Monoamine oxidase activity was determined using a modification of the procedure of Wurtman and Axelrod (1963). Rat brain tissues were homogenized in 6 volumes of distilled water. A portion (100 μl) of this homogenate was added to 400 μl of isotonic potassium chloride and vortexed, and 25 μl of this dilute homogenate was added to each tube (for blank controls 25 μl of potassium chloride was added instead). All tubes were placed on ice; to each was added 250 μl 0.5 M sodium phosphate buffer (pH=7.4). Aliquots (25 μl) of solutions of [^{14}C]-5HT (substrate for MAO-A) or [^{14}C]-phenylethylamine (substrate for MAO-B), appropriately diluted with respective unlabelled compounds, were added to each tube. Tubes were then incubated at 37°C for 20 min; after cooling to room temperature, 200 μl 2 M HCl was added to stop the reaction. Toluene (6 ml) was added to all tubes and the mixtures were vortexed for 5 min. After a brief centrifugation, the tubes were placed at -80°C until the aqueous layer was frozen. The toluene layer was decanted into a vial containing 9 ml of scintillation fluid. The mixtures were shaken thoroughly and radioactivity counted by liquid scintillation spectrometry. The amount of radioactivity in blank tubes was subtracted from all samples, and the values from sample vehicle controls averaged. The radioactivity in the sample was divided by that in controls and the value multiplied by 100 to give % activity. Percent inhibition was determined by subtracting % activities from 100.

J. ANALYSIS OF TRICYCLIC ANTIDEPRESSANTS

A modification of the procedure of Drebit *et al.* (1988) was used. Rat brain tissue was homogenized in 6 volumes of distilled water and a portion (2 ml) of this homogenate was used for analysis. Maprotiline [internal standard] (100 μl) was added to the homogenate which was centrifuged 1,000 x g for 10 min. The clear supernatant was collected and basified with solid sodium bicarbonate. Acetylation was then carried out using the procedure of Martin and Baker (1977). The acetylated DMI, maprotiline and underivatized IMI were extracted by shaking with ethyl acetate (5 ml) for 10 min on a vortex mixer. After a 5 min centrifugation at 1,000 x g, the organic phase was transferred to another set of tubes and evaporated to dryness under a stream of nitrogen. The samples were reconstituted by the addition of toluene (200 μl). A portion (1 μl) of this solution was injected onto a gas chromatograph equipped with a fused silica capillary column (see Section F) and a nitrogen-phosphorus detector. Chromatographic operating parameters were as follows: initial temperature 105°C; initial time 0.5 min; rate 25°C/min; final temperature 295°C; final time 5 min.

K. ANALYSIS OF PROTEIN CONCENTRATIONS

Protein concentrations in rat brain homogenates were determined according to Lowry *et al.* (1951). To an aliquot (50μl) of brain homogenate were added 750μl distilled water and 200μl of membrane digestor (1:1 v/v 1 N sodium hydroxide and 1% sodium deoxycholate). The mixture was vortexed and incubated at room temperature for 10 min, and 5 ml of reagent A (1/.01/.01 v/v/v 2% sodium carbonate, 1% cupric sulfate and 2% sodium potassium tartrate) were added, the tubes vortexed and incubated for another 10 min. Folin reagent (1:1 v/v 2 N folin and distilled water) [500μl] was then added, the tubes vortexed and incubated for a minimum of 30 min. A standard curve was run in parallel with the tissue samples, using bovine serum albumin as protein standard. All tubes were run through an ultraviolet visible spectrophotometer (wave length = 660 nm) to determine protein concentrations.

L. STATISTICAL ANALYSIS

Analysis of the data from functional tests involved non-parametric Kruskal-Wallis ANOVA, followed by Mann-Whitney U-tests where appropriate. All other analyses used parametric ANOVA followed by Newman-Keuls multiple comparisons when appropriate. A two-tailed probability distribution was used for all statistical analyses with the general convention of a probability criterion (p) of less than 0.05 used to establish statistical significance.

RESULTS AND DISCUSSION

A. DOSE-RESPONSE STUDIES ON THE GABA RECEPTOR AGONISTS BACLOFEN AND PROGABIDE.

Introduction: To determine the appropriate dose of baclofen and progabide for use in the functional tests, dose-response relationships for each GABA agonist were measured in separate groups of rats. In addition, to assess the stereoselectivity of the behavioural response to baclofen, both (±)- and (+)-baclofen were included. The range of drug doses chosen for analysis is consistent with previous behavioural studies of the effects of these compounds on locomotor activity (Di Scala *et al.*, 1985; Agmo and Giordano, 1985).

Results: Dose-response data for (±)- and (+)-baclofen (Fig. 11) and progabide (Fig. 12) are expressed as % control (vehicle) activity. Kruskal-Wallis ANOVA revealed that for both (±)-baclofen and progabide the degree of motor-suppression was significantly different from controls: [$H=34.6$, $p<0.05$] and [$H=19.6$, $p<0.05$], respectively. This suppression was significantly greater with each increasing drug dose. For (+)-baclofen, no effect on locomotor activity was observed at any of the tested doses [$H=2.0$, $p>0.05$].

Discussion: Based on the results from these dose-response studies, doses of 5 mg/kg and 50 mg/kg were chosen for use in the functional tests for (±)-baclofen and progabide respectively. The dose of 5 mg/kg (±)-baclofen represents the median dose and allowed for an accurate assessment of changes in its motor-suppressant effects, both increases and decreases, in the functional tests. Progabide at a dose of 50 mg/kg induced the most consistent reduction in locomotor activity of the two doses, and also provided the best

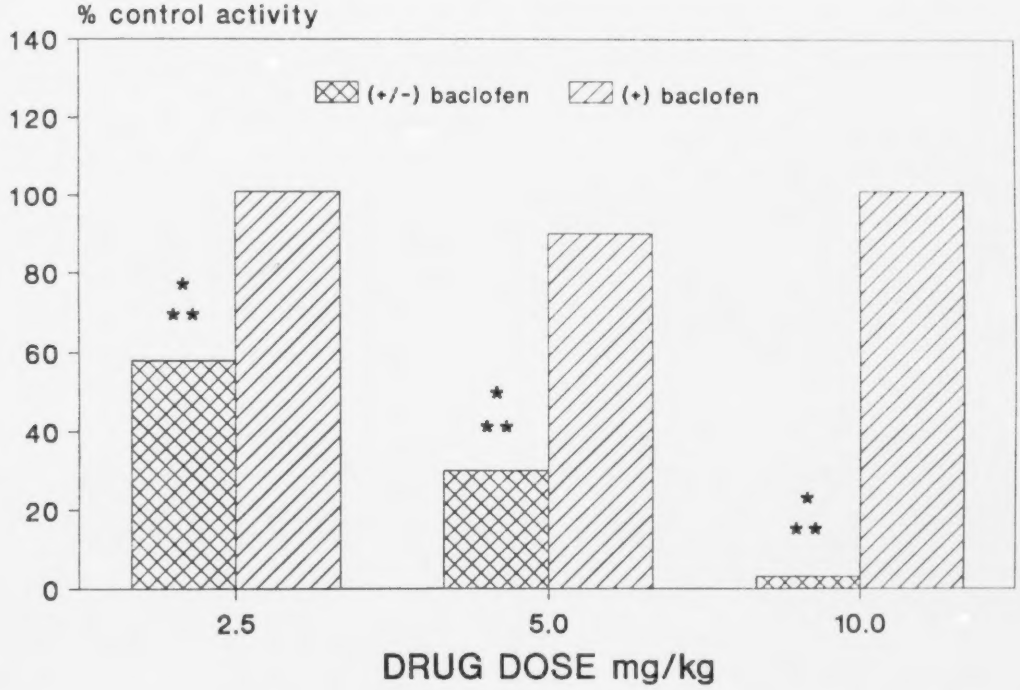


Figure 11: Dose-response data for (±)- and (+)-baclofen expressed as a % of activity exhibited by vehicle controls. * $p \leq 0.05$ compared to controls. Each dose effect was significantly different from each other (** $p \leq 0.05$). N=7-10 per treatment group.

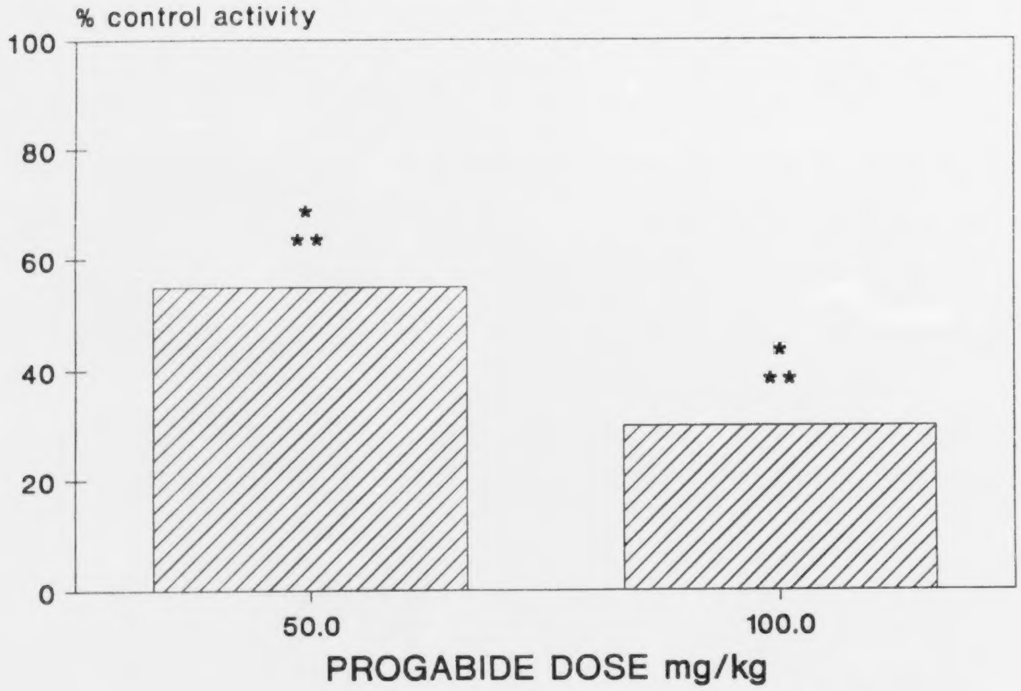


Figure 12: Dose-response data for progabide expressed as a % of activity exhibited by vehicle controls. * $p \leq 0.05$ compared to controls. The effects of the two doses were significantly different from each other (** $p \leq 0.05$). N=10-12 per treatment group.

activity level with which to estimate accurately any increases in its motor-suppressant effects. With respect to the stereoselectivity of the behavioural effects of baclofen, the lack of any effect of the (+)-baclofen on locomotor activity is in accord with the view that the (-) stereoisomer is the active compound (Chang *et al.*, 1982).

B. FUNCTIONAL ANALYSIS

B.1 *Effects of chronically administered antidepressant drugs on an in vivo behavioural test of β-adrenoceptor function.*

Introduction: Antidepressant drug-induced changes in β-adrenoceptors have been assessed both with *ex vivo* radioligand studies and *in vivo* behavioural tests of receptor sensitivity (Spyraki and Fibiger, 1980; Snyder and Peroutka, 1984; Maj *et al.*, 1984). With the *in vivo* technique, changes in the functional sensitivity of β-adrenoceptors are indicated by an alteration in the behavioural effects induced by selective receptor agonists (Przegalinski *et al.*, 1983, 1984; Baker and Greenshaw, 1989). An attenuation of the motor-suppressant effects of salbutamol [a β-adrenoceptor agonist] (Brittain *et al.*, 1968) has been found following chronic administration of a variety of antidepressant drugs, and was interpreted as behavioural evidence for the down-regulation of central β-adrenoceptors (Przegalinski *et al.*, 1983).

The present experiments were designed to assess the effects of chronic (21 d) administration of both MAO inhibitors (TCP, PLZ) and tricyclics (IMI, DMI) on the functional sensitivity of β-adrenoceptors to a challenge dose of salbutamol. Two doses each of PLZ and DMI were included to assess the possibility of dose-dependency in this context. The salbutamol response with β-adrenoceptors allowed for a comparison with published studies and also provided a comparative neurotransmitter system for an analysis of the effects of these same antidepressant drugs on $\mathrm{GABA_B}$ receptors.

Results: The degree of salbutamol-induced motor-suppression is expressed in Fig. 13 as a percentage of activity exhibited by each animal on the equivalent saline control day. For vehicle control animals, salbutamol reduced locomotor activity to 50% of that observed after saline injection. With each of the antidepressant drug groups, the response to salbutamol was significantly attenuated or completely abolished [$H=14.9$, $p<0.05$]. For PLZ, the effect was dose-dependent, with only the 10 mg/kg/d dose attenuating the motor-suppressant effect of salbutamol. Although the average effect of DMI at 5 mg/kg/d on the salbutamol response was apparently reduced compared to the higher dose (see Fig. 13), the difference did not reach statistical significance. Saline day locomotor activity was reduced by chronic antidepressant drug treatments compared to vehicle controls (see Appendix II).

Discussion: The present results are in agreement with previous reports of decreased functional sensitivity of β-adrenoceptors following chronic antidepressant treatments (Maj *et al.*, 1984). In addition, these results are consistent with the down-regulation of β-adrenoceptors based on radioligand binding studies (Banerjee *et al.,* 1977; Wolfe *et al.,* 1978) and β-adrenoceptor-linked adenylate cyclase activity measurements (Vetulani and Sulser, 1975). An induction of functional subsensitivity of β-adrenoceptors has not previously been demonstrated for the MAO inhibitors TCP and PLZ. The observation of the dose-dependent effect of PLZ on the behavioural response to salbutamol is a robust phenomenon, having recently been replicated (Paetsch and Greenshaw, unpublished) using the same behavioural paradigm.

Because β-adrenoceptors were used as a reference for comparison with the GABA receptors, only those doses of antidepressants that reduced the behavioural response to salbutamol were included in the subsequent assessment of changes in

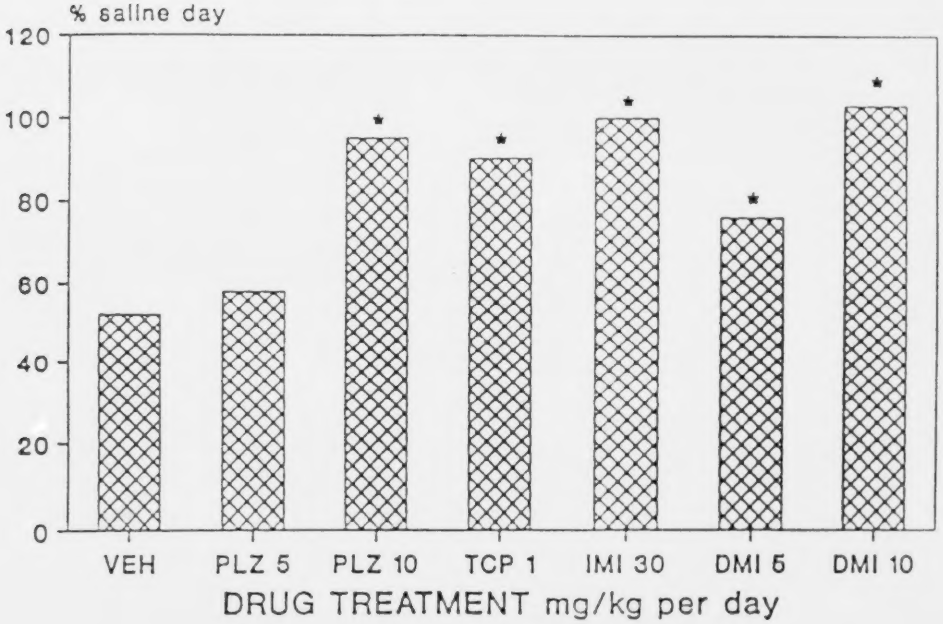


Figure 13: Effects of chronically administered antidepressant drugs on the degree of salbutamol-induced suppression of locomotor activity expressed as a % of activity exhibited on the equivalent saline control day. * $p \leq 0.05$ compared to vehicle controls. N=8-10 per treatment group.

$GABA_B$ receptor function, GABA and β-adrenergic binding and indices of GABA metabolism. Expressed as mg/kg/d, salt doses, drug doses chosen for study were as follows: PLZ, 10; TCP, 1; IMI, 30; and DMI, 10. The higher 10 mg/kg/d dose of DMI was used because it induced a more robust decrease in β-adrenoceptor responsiveness than did the 5 mg/kg/d dose.

B.2 *Effects of chronically administered antidepressants on an in vivo behavioural test of $GABA_B$ receptor function.*

Introduction: Following the proposal by Lloyd *et al.* (1985) that an increase in the number of $GABA_B$ binding sites was a general consequence of long-term antidepressant treatment, several *ex vivo* assays were employed to assess the effect of this receptor change on measures of $GABA_B$ function. The most commonly used assays involved measurement of changes in the effect of baclofen on cAMP accumulation and K^+-evoked release of 5-HT in cortical slices (Suzdak and Gianutsos, 1986; Gray and Green, 1987). Relatively few studies have, however, determined the functional significance of the purported $GABA_B$ receptor changes *in vivo*. Using the same behavioural paradigm as for the salbutamol response on β-adrenoceptors, the present study assessed the effect of chronic (21 d) administration of IMI, DMI, TCP and PLZ on the functional sensitivity of $GABA_B$ receptors *in vivo*. (±)-Baclofen and progabide were used as GABAergic agonists. It had been hypothesized that an antidepressant-induced increase in the number of $GABA_B$ receptors would result in increased behavioural effects of the GABA agonists.

Results: The degree of (±)-baclofen (Fig. 14) and progabide (Fig. 15)-induced motor-suppression is expressed as a % of activity exhibited by each animal on the equivalent saline control day. In contrast to the reduced behavioural effects observed with

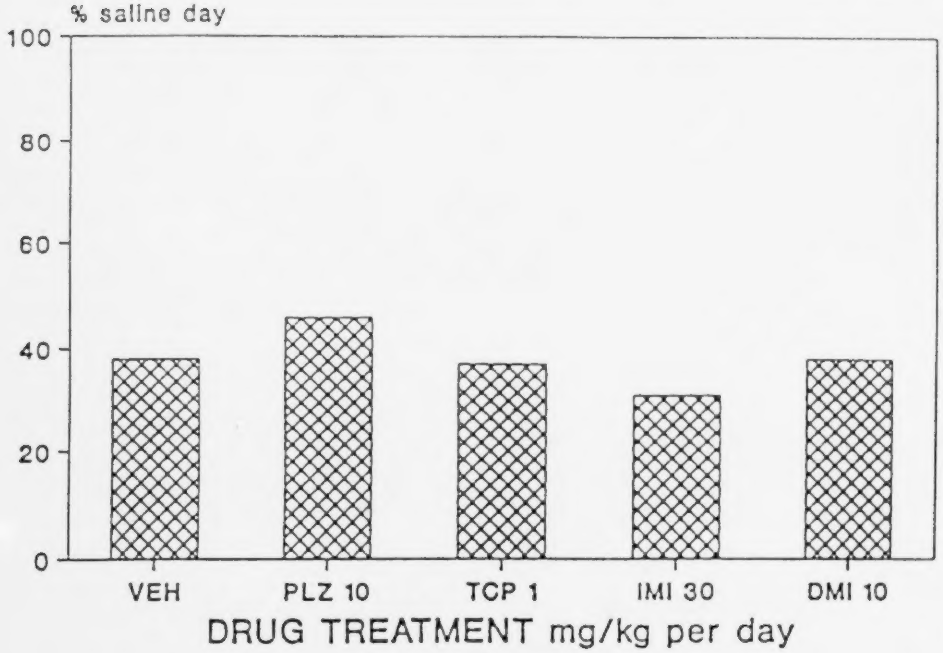


Figure 14: Effects of chronically administered antidepressant drugs on the degree of (±)-baclofen-induced suppression of locomotor activity expressed as a % of activity exhibited on the equivalent saline control day. N=8-10 per treatment group.

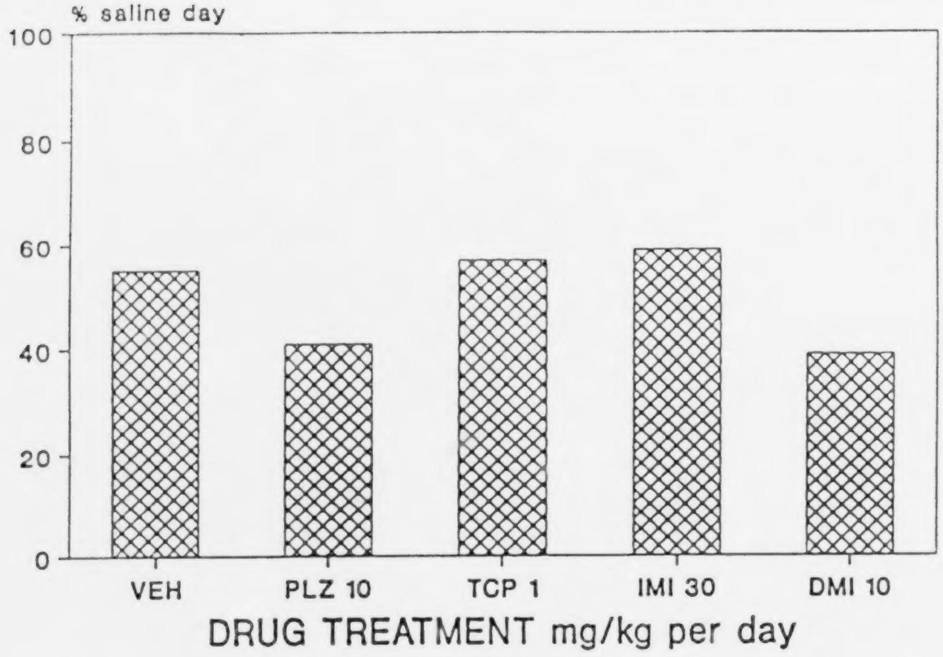


Figure 15: Effects of chronically administered antidepressant drugs on the degree of progabide-induced suppression of locomotor activity expressed as a % of activity exhibited on the equivalent saline control day. N=8-10 per treatment group.

salbutamol, the motor-suppressant actions of (±)-baclofen and progabide were not found to be significantly altered by any of the antidepressant treatments [$H=3.8$, $p>0.05$; $H=1.7$, $p>0.05$, respectively]. In both vehicle and drug treatment groups, locomotor activity was reduced to the same degree by the GABA agonists. Saline day locomotor activity was reduced by chronic antidepressant drug treatments compared to vehicle controls (see Appendix II) with both GABA agonist tests.

Discussion: Previous *in vivo* functional studies of the effects of chronic antidepressant treatment have produced evidence both for and against an increase in the number of $GABA_B$ receptors. Gray *et al.* (1987) found that repeated treatment with both tricyclic antidepressants and electroconvulsive shocks significantly enhanced the hypothermic response to baclofen, consistent with an increase in $GABA_B$ receptor function. Borsini *et al.* (1986), however, observed that baclofen-induced antinociception was not affected by chronic administration of the tricyclic DMI. In parallel with the finding of Borsini *et al.*, the present behavioural test also failed to indicate any increases in $GABA_B$ receptor function. Chronic administration of antidepressants both MAO-inhibitors and tricyclics failed to modify the behavioural effects of the GABA agonists (±)-baclofen and progabide. Under identical experimental conditions the antidepressants did, however, induce a functional down-regulation of β-adrenoceptors. These data do not support the proposal of a functional increase in the number of $GABA_B$ receptors following chronic antidepressant treatment.

It has been previously demonstrated that progabide is a mixed $GABA_A$/$GABA_B$ receptor agonist (Lloyd *et al.*, 1982). As such, the behavioural effects of progabide cannot simply be ascribed to a singular action at $GABA_B$ receptors. This point is of particular interest, considering several studies which indicate that chronic antidepressants may down-regulate the $GABA_A$/benzodiazepine receptor complex (Suranyi-Cadotte *et al.*, 1984; Barbaccia *et al.*, 1986; Borsini *et al.*, 1986). It is therefore possible that increases in the behavioural effects of progabide could have been offset by the effect of down-regulation of the $GABA_A$/benzodiazepine receptor complex. Two findings from the present data argue against this possibility: (1) failure to observe any change in the behavioural effects of the $GABA_B$ specific agonist (±)-baclofen, (2) given the lack of effect on $GABA_B$ receptors, the presence of down-regulated $GABA_A$ receptors, should have resulted in a reduced behavioural effect of progabide. This was not observed.

Selectivity for $GABA_B$ receptor mediation in the behavioural response to (±)-baclofen is supported by the findings of Agmo and Giordano (1985) who demonstrated that the reduction of locomotor activity induced by (±)-baclofen and by the GABA-T inhibitor γ-acetylenicGABA (GAG) was not affected by the $GABA_A$ antagonist bicuculline. These authors concluded that the motor-suppressant effects were mediated by $GABA_B$ receptors and that the $GABA_A$ receptor was not important for the locomotion-reducing effects of GABAergic drugs. Further support for a $GABA_B$ receptor selectivity is indicated by the finding that the (+) enantiomer of baclofen, which is inactive at GABA receptors, did not induce any behavioural effects in the present test (see Fig. 11).

It must be noted that with these *in vivo* behavioural tests of both β-adrenergic and $GABA_B$ receptor function, it is not possible to discriminate between changes in receptor affinity (Kd) or number (Bmax) that might be influencing the functional response.

C. RECEPTOR BINDING ANALYSIS: EFFECTS OF CHRONICALLY ADMINISTERED ANTIDEPRESSANT DRUGS

C.1 *β-Adrenergic Binding Sites*

Introduction: The second component of this thesis project involved the *ex vivo* analysis of the effects of chronic antidepressants on radioligand binding assays. Consistent with the strategy employed in the functional tests, β-adrenoceptors were again used as the comparative neurotransmitter system. First established by Banerjee *et al.* (1977), decreased [^{3}H]-DHA binding to β-adrenergic binding sites has been shown to be a reliable emergent change following chronic antidepressant drug therapy (Maj *et al.*, 1984; Bergstrom and Kellar, 1979; Sellinger-Barnette *et al.*, 1980). Two doses, 5 and 10 mg/kg/d, of PLZ were included in the [^{3}H]-DHA binding study to determine whether a change in β-adrenergic binding site affinity or number might underlie the dose-dependent effects seen with PLZ in the previous behavioural test of β-adrenoceptor function. Antidepressants were administered for 28 consecutive days before all binding analyses.

Results: The effects of chronic antidepressant treatment on [^{3}H]-DHA binding parameters are displayed in Table 7. From these data, it is evident that all drug treatments induced a significant reduction in the number (B_{max}) of binding sites [$F(5,52)=13.136, p<0.05$]. There was no effect of the drugs on binding site affinity (K_d) [$F(5,52)=1.778, p>0.05$]. Scatchard and Ligand analysis of the binding isotherms revealed a single population of binding sites with Scatchard linear correlations between 0.98 and 0.99. Hill plot analysis provided no indication for cooperativity of binding,

Drug Treatment	Dose mg/kg/d	K_d nM	B_{max} fmol/mg Protein
Vehicle		1.04 ± 0.10	92.6 ± 3.7
PLZ	5	0.94 ± 0.13	70.6 ± 3.2*
PLZ	10	0.94 ± 0.15	69.1 ± 4.2*
TCP	1	0.84 ± 0.07	73.6 ± 3.8*
IMI	30	0.94 ± 0.16	57.6 ± 3.1*
DMI	10	1.46 ± 0.29	65.3 ± 2.6*

Table 7: Effects of chronic antidepressant drug treatment on [^{3}H]-DHA binding in cerebral cortex. Values are mean ± SEM, N=8 saturation curves. * $p \leq 0.05$ compared to vehicle controls.

with Hill coefficients of 1.0 ± 0.1. Control values for [^{3}H]-DHA binding B_{max} (92.6 ± 3.7 fmol/mg protein) and K_d (1.04 ± 0.1 nM), were in good agreement with previous binding studies (Banerjee *et al.*, 1977; Bylund and Snyder, 1976). There was no difference in the B_{max} estimates for PLZ at 5 and 10 mg/kg/d. Both doses reduced [^{3}H]-DHA binding by approximately 25% compared to vehicle controls. A representative Scatchard plot of [^{3}H]-DHA binding is shown in Fig. 16.

Discussion: The present radioligand binding study confirms and extends the literature describing a common reduction in the number of β-adrenergic binding sites as a consequence of long-term antidepressant treatment (Frazer *et al.*, 1974; Clements-Jewery, 1978; Campbell *et al.*, 1979a,b; Hall *et al.*, 1980; Maj *et al.*, 1984). A comparison of these binding changes with results from the β-adrenoceptor functional tests reveals that, in general, there was good agreement between the two measures: doses of antidepressants that induced β-adrenoceptor subsensitivity also significantly reduced the density of β-adrenergic binding sites (see Fig. 13 and Table 7). A dissociation between changes in β-adrenergic binding and receptor sensitivity was, however, found for the effects of PLZ at 5 and 10 mg/kg/d. Both doses significantly reduced [^{3}H]-DHA binding, but only the higher dose of PLZ induced a change in the behavioural measure of β-adrenoceptor sensitivity. This result indicates that the dose-dependent effect of PLZ observed in the functional test cannot be adequately accounted for on the basis of a differential dose effect on receptor number, and suggests that other neuronal modifications in addition to a reduction in receptor number were operative in the induction of functional changes in β-adrenoceptors. Potential processes involved include a post-receptor change in the coupling of β-adrenoceptors to the adenylate cyclase signal transduction pathway, and/or mediation through increased modulatory activity of the endogenous trace amine β-phenylethylamine. This second process will be discussed in Section E. With respect

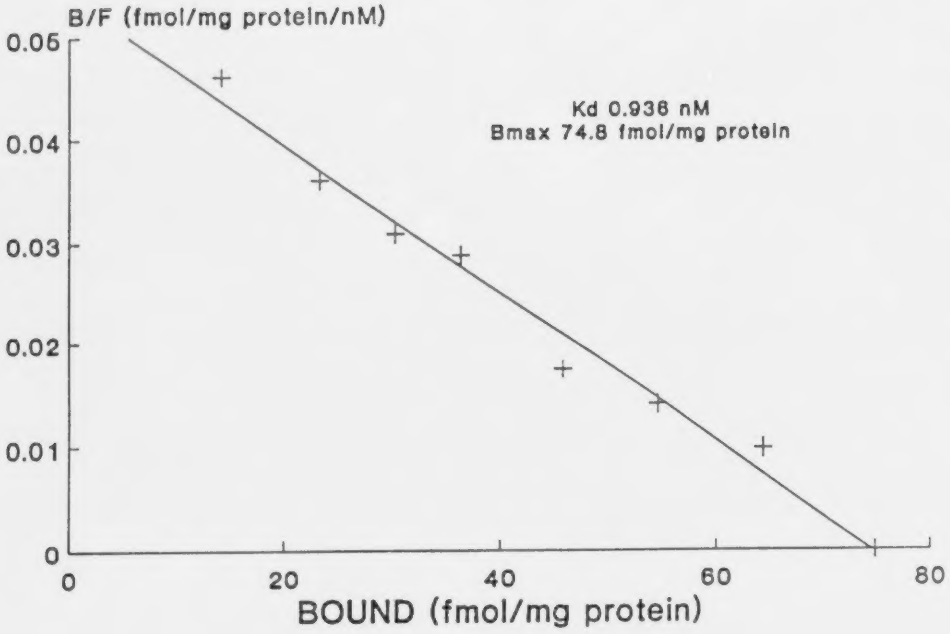


Figure 16: A representative Scatchard plot of [^{3}H]-DHA binding to β-adrenergic binding sites.

to the binding procedure itself, the use of an antagonist ([^{3}H]-DHA in this case) as radiolabelled ligand results in an inability to detect changes in agonist affinity states, which could significantly influence receptor function *in vivo*.

A lack of correspondence between the binding and behavioural effects of PLZ on β-adrenoceptors illustrates the primary limitation of radioligand binding studies: their lack of information about the functional significance of the binding change. For this reason, all analyses of emergent receptor changes and their potential role in the therapeutic response to antidepressant treatments must include some functional measure of receptor change, either *in vivo* or *ex vivo*, in addition to any receptor binding measurements.

C.2 *GABA$_B$ Binding Sites*

Introduction: An important aim in carrying out radioligand binding analysis of the effects of chronic antidepressant drugs on GABA$_B$ binding sites was to attempt to replicate and extend the work of Lloyd *et al.* (1985). As outlined earlier, these researchers demonstrated that repeated administration of antidepressants of every drug class, as well as electroconvulsive shocks, all produced a large increase in the number of GABA$_B$ binding sites in the frontal cortex. Subsequent work on GABA$_B$ binding changes has, however, produced a mixture of supportive and contradictory results. The present binding study was designed to investigate the generality of this binding change and involved an analysis of the effects of chronic (28 d) administration of antidepressants on GABA$_B$ binding. Included in the study were one tricyclic (DMI) studied by Lloyd *et al.* (1985) and essentially the same binding protocol as used by these workers. An additional aim of this binding study was to compare the effects of two tricyclics (IMI and DMI) that have been assessed for their effect on GABA$_B$ binding with two MAO inhibitors (PLZ and TCP) that have not previously been studied in this context.

Results: Table 8 displays the effects of repeated antidepressant drug treatment on [^{3}H]-GABA binding to GABA$_B$ binding sites. In contrast to the action of these antidepressants on [^{3}H]-DHA binding, [^{3}H]-GABA binding was not found to be significantly altered. ANOVA revealed that neither the B$_{max}$ [$F(4,25)=1.963$, $p>0.05$] nor K$_d$ [$F(4,25)=1.998$, $p>0.05$] of the drug groups was significantly different from vehicle controls. TCP-treated animals showed a tendency towards increased [^{3}H]-GABA binding, but the differences did not reach statistical significance. The [^{3}H]-GABA binding data were further analyzed by subjecting the values for specific binding at a concentration corresponding to the K$_d$ to ANOVA. This analysis, in accord with the results of the B$_{max}$ estimates, revealed no effect of antidepressant drugs on GABA$_B$ binding [$F(4,25)=2.58$, $p>0.05$]. Ligand and Hill plot analysis of binding isotherms indicated a single population of binding sites with no evidence for binding cooperativity. B$_{max}$ and K$_d$ estimates for [^{3}H]-GABA binding in control animals (709 ± 73 fmol/mg protein and 50.1 ± 5.8 nM respectively) were in good agreement with previous studies of high affinity GABA$_B$ binding (Lloyd *et al.*, 1985; Szekely *et al.*, 1987). A representative Scatchard plot of [^{3}H]-GABA binding is shown in Fig. 17.

Discussion: Repeated (28 d) administration of IMI, DMI, TCP and PLZ, four clinically effective antidepressants, did not significantly alter the number of GABA$_B$ binding sites in rat frontal cortex. These results are not in agreement with the marked increase in GABA$_B$ binding reported by Lloyd *et al.* (1985). Methodological differences in binding procedures cannot easily account for the discrepancy in results. In both studies, antidepressants were administered by subcutaneous infusion using osmotic minipumps. Frontal cortex membrane preparations were both prepared according to Hill and Bowery (1981). Finally, the binding assays utilized the same radioligand ([^{3}H]-GABA) and unlabelled displacer drug (GABA) and determined non-specific binding with the same compound [(±)-baclofen]. One procedural difference between the two studies

Drug Treatment	**Dose** **mg/kg/d**	**K_d** **nM**	**B_{max}** **fmol/mg Protein**
Vehicle		50.1 ± 5.8	709 ± 73
PLZ	10	46.4 ± 10.6	838 ± 153
TCP	1	67.2 ± 6.6	1183 ± 146
IMI	30	38.8 ± 5.9	851 ± 107
DMI	10	52.5 ± 7.1	909 ± 131

Table 8: Effects of chronic antidepressant drug treatment on $[^3H]$-GABA$_B$ binding in frontal cortex. Values are mean ± SEM, N=6 saturation curves.

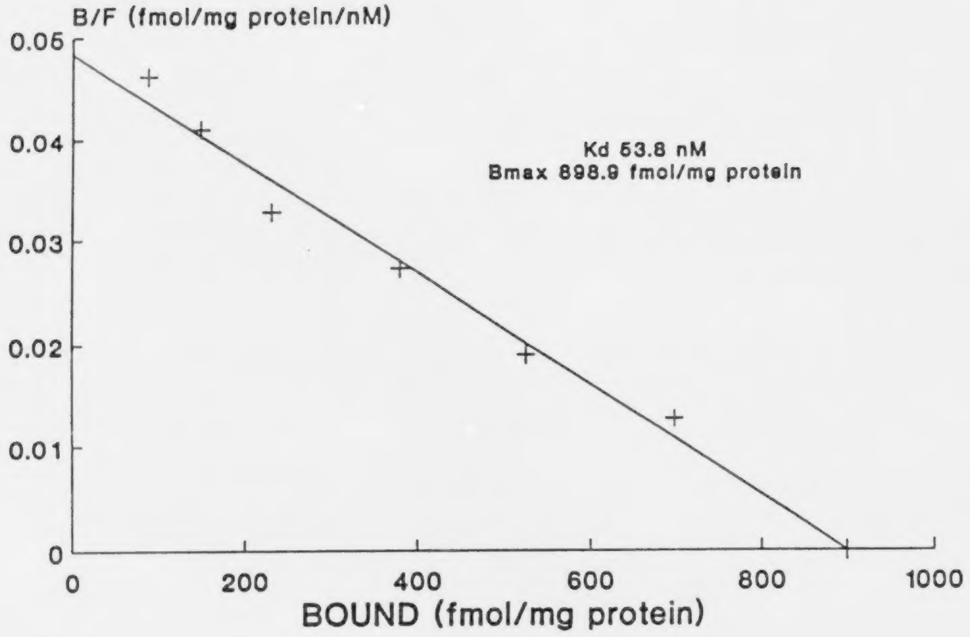


Figure 17: A representative Scatchard plot of [^{3}H]-GABA binding to GABA$_B$ binding sites.

was that Lloyd *et al.* (1985) killed their animals 72 h after the last dose of drug, whereas in the present study animals were killed immediately following 28 d administration of the antidepressants. Based on the findings of a study by Cross and Horton (1988), this time difference is not likely to have had any effect on the discrepant binding results. These authors demonstrated that $GABA_B$ binding site density following chronic DMI administration, when compared to controls, did not differ whether the animals were killed 24 h or 72 h after cessation of drug delivery. In addition, as none of the administered antidepressants has any appreciable affinity for the $GABA_B$ receptor, it is unclear why this 72 h delay before dissection was included by Lloyd *et al.* (1985).

The finding of a lack of effect of repeated antidepressants on $GABA_B$ binding is consistent with the results of several previous studies (Cross and Horton, 1987, 1988; Szekely *et al.*, 1987). It is worth noting that in the study by Szekely *et al.* (1987) no change in $GABA_B$ binding was found when $[^3H]$-baclofen was used to label receptors but that an increased amount of binding was found when $[^3H]$-GABA was used. A dissociation between $[^3H]$-baclofen and $[^3H]$-GABA binding is in agreement with the results of Drew *et al.* (1984), who demonstrated that (-)-baclofen may not be able to displace all specifically bound GABA in these binding studies, and is consistent with GABA binding to sites other than those labelled by baclofen (Johnston, 1986). Scherer *et al.* (1988) have recently provided evidence for a multiplicity of pharmacologically distinct $GABA_B$ recognition sites. Taken together, these findings which indicate the existence of subtypes of $GABA_B$ receptors may help explain the variable $GABA_B$ functional test results. Whether the existence of multiple $GABA_B$ receptors can also provide an answer for the variable $[^3H]$-baclofen and $[^3H]$-GABA binding changes seen in studies using virtually identical binding procedures is unclear. The development and testing of selective $GABA_B$ agonists and antagonists like phaclofen, saclofen and 2-hydroxysaclofen, will be of great value in assessing this question.

In the present study [^{3}H]-GABA$_B$ binding changes were assessed in frontal cortical tissue. This tissue was chosen for study because it allowed for a comparison to be made between the present results and those from previous studies. It is unlikely, however, that possible chronic antidepressant effects on GABA$_B$ receptors would be restricted to the frontal cortex. Indeed, Lloyd *et al.* (1985) found GABA$_B$ receptor density to be altered in both rat frontal cortex and hippocampus following chronic DMI and other antidepressants. To date, no comprehensive study has looked at regional brain GABA$_B$ binding changes following chronic antidepressant treatments. A tissue selectivity for antidepressant effects on GABA$_B$ receptors is also unlikely given the systemic administration of the antidepressants.

In conclusion, it was observed that chronic administration of representative tricyclics (IMI, DMI) and MAO inhibitors (TCP, PLZ) significantly reduced the number of β-adrenergic binding sites, but did not alter the number of GABA$_B$ binding sites. This finding, together with the conflicting binding and functional test results, suggests that the influence of antidepressants on GABA$_B$ receptors is more complex than previously thought, and does not support the proposal that an increase in the total number of cortical GABA$_B$ receptors is a common effect of repeated treatment with antidepressants.

C.3 *GABA$_A$/Benzodiazepine Binding Sites*

Introduction: Repeated administration of antidepressant drugs has been shown to either have no effect (Lloyd and Pilc, 1984a; Kimber *et al.,* 1987) or to down-regulate (Suranyi-Cadotte *et al.,* 1984; Barbaccia *et al.,* 1986) various aspects of the GABA$_A$/benzodiazepine receptor complex. These studies have, however, been carried out almost exclusively with tricyclics and novel antidepressants, with very little attention paid to the effect(s) of MAO inhibitors. With the previous finding from Section C.2

that none of the tested antidepressants (including the MAO inhibitors TCP and PLZ) significantly altered $GABA_B$ binding, it was of particular interest to determine whether these drugs would induce any change in the number and/or affinity of $GABA_A$/benzodiazepine binding sites. For this analysis, binding of the radiolabelled $GABA_A$ receptor agonist muscimol was measured in rat cerebral cortex. It had been hoped that both high and low affinity muscimol binding components could be measured, but due to the very rapid ligand dissociation kinetics for the low affinity site, and the relatively long termination step provided even by rapid filtration on a cell harvester, it was only possible to obtain accurate estimates for the high affinity binding component. The results of this determination are detailed below.

Results: Chronic antidepressant drug effects on $[^3H]$-muscimol binding characteristics are displayed in Table 9. Neither the B_{max} $[F(4,28)=0.676, p>0.05]$ nor K_d $[F(4,28)=0.355, p>0.05]$ of high affinity $[^3H]$-muscimol binding was altered by any of the antidepressant drugs. Ligand and Hill analysis of the binding data indicated a single population of high affinity binding sites with no cooperativity of binding. A representative Scatchard plot is shown in Fig. 18. Control values for K_d (9.7 ± 0.9 nM) and B_{max} (697 ± 64 fmol/mg protein) are in good agreement with literature values (Ito *et al.*, 1988).

Discussion: In the present study, repeated (28 d) administration of IMI, DMI, TCP and PLZ failed to alter high affinity $[^3H]$-muscimol binding. This result does not replicate the findings of a previous study by Suzdak and Gianutsos (1985a) who observed that chronic administration of either IMI or nomifensine induced a significant reduction in the density of both the high and low affinity $[^3H]$-$GABA_A$ binding sites in mouse cerebral cortex. The present results are, however, consistent with two studies by Lloyd and Pilc (Lloyd and Pilc, 1984b; Pilc and Lloyd, 1984) which described a lack of effect of antidepressant drug treatments on $[^3H]$-$GABA_A$ binding. In these studies by Lloyd

Drug Treatment	**Dose mg/kg/d**	**K_d nM**	**B_{max} fmol/mg Protein**
Vehicle		9.7 ± 0.9	697 ± 64
PLZ	10	10.5 ± 1.0	635 ± 57
TCP	1	9.7 ± 1.0	583 ± 32
IMI	30	10.1 ± 0.8	649 ± 59
DMI	10	9.1 ± 0.5	589 ± 64

Table 9: Effects of chronic antidepressant drug treatment on [^{3}H]-muscimol binding in cerebral cortex. Values are mean ± SEM, N=6-7 saturation curves.

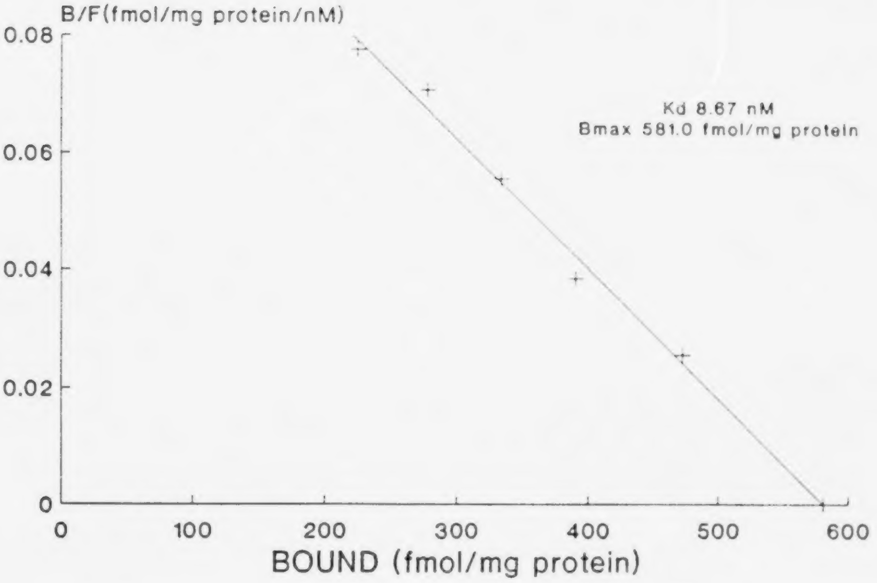


Figure 18: A representative Scatchard plot of [^{3}H]-muscimol binding to GABA$_A$ binding sites.

and Pilc, there was no indication of whether a separation between high and low affinity [^{3}H]-GABA$_A$ components was made in their analysis. This point is of particular relevance because it is generally thought that it is the lower affinity GABA$_A$ sites that are functionally linked to operation of the GABA$_A$/benzodiazepine receptor complex (Unnerstahl *et al.*, 1981). Based on these facts, the most appropriate conclusion from the [^{3}H]-muscimol binding data would be that they provide no evidence for an effect of repeated administration of antidepressants on high affinity GABA$_A$ binding sites. In addition, many studies investigating potential antidepressant-induced changes to the GABA$_A$ receptor complex involve a measurement of changes in the binding characteristics of the associated benzodiazepine binding site (Suranyi-Cadotte *et al.*, 1984; Barbaccia *et al.*, 1986; Kimber *et al.*, 1987).

The finding that chronic administration of PLZ did not alter [^{3}H]-muscimol binding is intriguing. As will be shown in Section D and in accord with previous studies (Popov and Matthies, 1969; Baker *et al.*, 1991) repeated PLZ treatment induces a significant and long-lasting elevation in brain GABA levels. An increase in the levels of a receptor agonist would be expected to result in a concomitant decrease in the number of binding sites for that agonist. An obvious assumption of this statement, however, is that the elevated agonist levels must be in an area (e.g. synaptic cleft) where they can interact with the binding sites. It would appear that for the PLZ-induced GABA increase this is not the case. It is tempting to speculate that the existence of the large number of GABA uptake sites on glial cells (Iversen and Kelly, 1975), in addition to those on presynaptic terminals (Martin, 1976), may account for the lack of effects on binding. With such rapid and efficient removal there would be little opportunity for the GABA to interact with the binding sites.

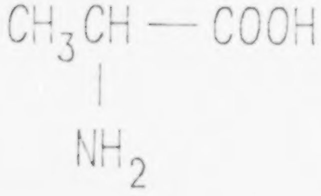
D. EFFECTS OF CHRONICALLY ADMINISTERED ANTIDEPRESSANT DRUGS ON BRAIN GABA METABOLISM

Introduction: Numerous clinical studies have provided evidence for an alteration in GABA metabolism in depressed patients. Changes previously reported include decreased GABA levels in both cerebrospinal fluid and plasma (Berrettini *et al.*, 1983; Petty and Schlesser, 1981) and reduced GAD activity in several brain regions (Perry *et al.*, 1977). Preclinical investigations have revealed that antidepressant drugs can modify GABA release acutely (Korf and Venema, 1983) and interfere with its degradation when administered acutely or chronically (Popov and Matthies, 1969). Based on these findings, it was of interest to determine what effects the present chronically tested antidepressants had on rat brain GABA metabolism. For this purpose, 3 measures of GABA metabolism were made: (1) GABA levels, (2) GAD activity and (3) GABA-T activity. Along with GABA, the levels of 4 other aliphatic amino acids of interest to biological psychiatry were analyzed. The amino acids were alanine (Ala), valine (Val), leucine (Leu) and isoleucine (Ileu) [see Fig. 19]. All amino acid levels and enzyme activity measurements were from frontal cortex, so that estimates of both $GABA_B$ binding and GABA metabolism were from the same tissue.

Results: Brain concentrations for the amino acids listed in Table 10 are in good agreement with reported literature values (Suñol *et al.*, 1988; Yeung *et al.*, 1986; Wong *et al.*, 1990a). As can be seen, no effect was found on any of the amino acid levels following IMI, DMI or TCP treatment (see Appendix I for statistics). In contrast, chronic PLZ induced a significant elevation in both GABA [$F(4,30)=9.62$, $p<0.05$] and Ala [$F(4,30)=3.83$, $p<0.05$] levels. Levels of the other amino acids were not altered by

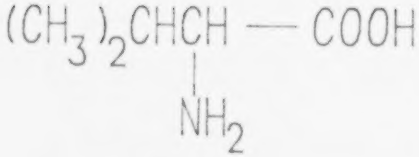
$H_2N—CH_2CH_2CH_2—COOH$

GABA



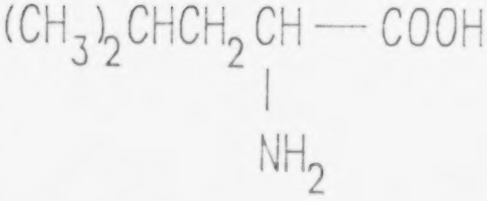
$CH_3CH(NH_2)—COOH$

Alanine



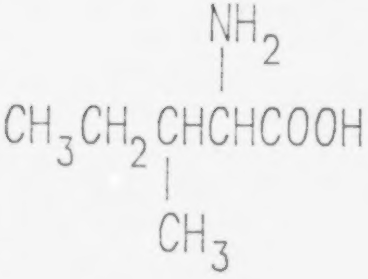
$(CH_3)_2CHCH(NH_2)—COOH$

Valine



$(CH_3)_2CHCH_2CH(NH_2)—COOH$

Leucine



$CH_3CH_2CH(CH_3)CH(NH_2)COOH$

Isoleucine

Figure 19: Structures of amino acids analyzed in this study.

Amino Acid Levels (μg/g brain tissue)

Drug Treatment	Dose mg/kg/d	GABA	Ala	Val	Leu	Ileu
Vehicle		237.8 ± 10.1	50.6 ± 4.1	9.5 ± 0.9	7.2 ± 0.4	3.8 ± 0.3
PLZ	10	*366.2 ± 30.2	*79.7 ± 11.1	9.5 ± 1.0	8.1 ± 0.7	4.2 ± 0.4
TCP	1	254.4 ± 14.1	58.4 ± 6.3	10.6 ± 0.9	8.1 ± 0.5	5.5 ± 1.0
IMI	30	224.5 ± 13.5	47.1 ± 4.7	9.2 ± 0.5	6.3 ± 0.8	3.6 ± 0.2
DMI	10	247.8 ± 17.3	49.1 ± 5.7	9.9 ± 1.4	7.7 ± 0.9	4.2 ± 0.6

Table 10: Effects of chronic antidepressant drug treatment on amino acid levels in frontal cortex. Levels are mean ± SEM, N=7. * $p \leq 0.05$ compared to vehicle controls.

PLZ treatment. Representative gas chromatograms of derivatized amino acids are shown in Fig. 20. Resultant standard curves were linear (correlation coefficients > 0.99) in the concentration range 30-1000 ng for all the amino acid derivatives.

Of the antidepressants tested, only PLZ treatment was found to influence measures of GABA metabolism. Chronic IMI, DMI or TCP did not affect either GAD or GABA-T activity, while PLZ caused a marked reduction in GABA-T activity [Table 11]. GAD activity was also moderately reduced by PLZ but the difference was not statistically significant. ANOVA values for GAD and GABA-T were [$F(4,31)=0.71$, $p>0.05$] and [$F(4,31)=4.74$, $p<0.05$], respectively. GAD and GABA-T activities expressed as μmol/g/h are consistent with previous literature values (Albers and Brady, 1958; Sterri and Fonnum, 1978).

Discussion: The present set of experiments was carried out to determine what effect(s) chronically administered antidepressant drugs had on different indices of GABA metabolism. It was observed that neither IMI nor DMI had any appreciable influence on GABA levels, GAD activity or GABA-T activity. This result confirms and extends the findings of a study by Pilc and Lloyd (1984) where no effect on GAD activity was found following 18 d treatment with several tricyclic antidepressants including amitriptyline, citalopram, viloxazine and DMI. Tricyclic antidepressants have, however, been shown to influence GABA synapses when administered acutely. Korf and Venema (1983) and Korf *et al.* (1981) demonstrated a marked increase in GABA release from rat striatum and thalamus following acute IMI, DMI and trimipramine perfusion. It would seem that although tricyclic antidepressants can have acute effects on GABA synaptic activity, a significant effect on GABA metabolism is not likely to play a prominent role in their mode(s) of action. This conclusion does not appear to hold for at least some types of MAO inhibitors. Chronic treatment with PLZ, but not with TCP, induced a significant elevation in GABA levels (54% above controls) and inhibition of

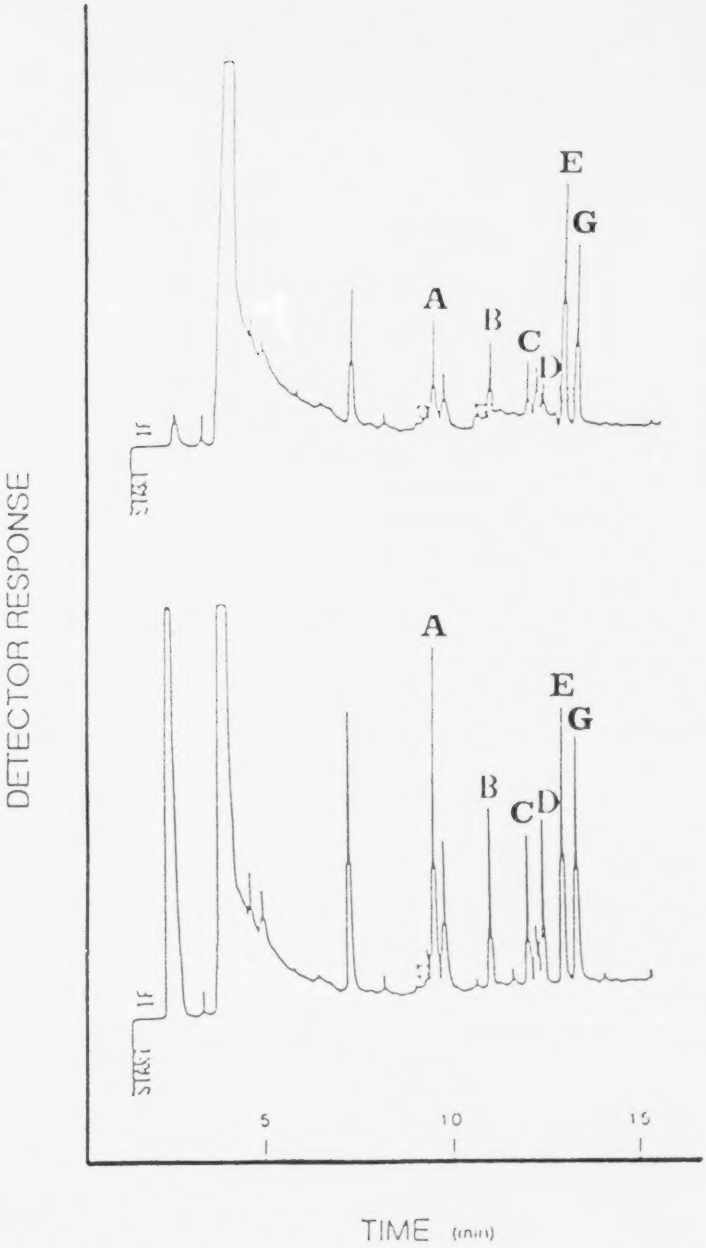


Figure 20: Gas chromatograms of derivatized amino acids from homogenized rat brain (top) and authentic standards (bottom). Peaks are: A=Ala; B=Val; C=Leu; D=Ileu; E=Nleu (internal standard); and G=GABA. Reprinted with permission by Dr. J.T.F. Wong.

Drug Treatment	**Dose mg/kg/d**	**GAD Activity**	**GABA-T Activity**
Vehicle		8.0 ± 1.3	26.6 ± 2.9
PLZ	10	5.9 ± 0.9	19.2 ± 1.2*
TCP	1	8.0 ± 1.0	28.5 ± 1.2
IMI	30	7.0 ± 0.9	28.3 ± 1.4
DMI	10	7.3 ± 1.0	27.4 ± 1.6

Table 11: Effects of chronic antidepressant drug treatment on frontal cortex GAD and GABA-T activities. Enzyme activity is mean ± SEM, N=7. * $p \leq 0.05$ compared to vehicle controls.

GABA-T activity (28% below controls) in frontal cortex. The effects of PLZ on GABA metabolism are thought to be due to the action of the hydrazine moiety present in the PLZ molecule. Hydrazines are carbonyl-trapping compounds and inhibit GABA-T by interfering with co-factor (pyridoxal phosphate) [Roberts, 1986] availability for the transamination reaction (Tunnicliff, 1989). Results from a comprehensive study by Popov and Matthies (1969) suggest that the ability of PLZ to increase GABA levels is not simply the result of an interference with pyridoxal phosphate. These researchers demonstrated that pretreatment of rats with the pyridoxal phosphate precursor pyridoxine at high doses did not alter the inhibition of GABA-T produced by PLZ. They did, however, show that pretreatment of rats with TCP (which inhibits MAO, but does not affect GABA or GABA-T) completely abolished the inhibitory effect of PLZ on GABA-T. Based on these two findings, together with the previous observation that PLZ is both a substrate for, and inhibitor of MAO (Horita, 1965; Clineschmidt and Horita, 1968), Popov and Matthies (1969) suggested that a metabolite of PLZ (presumably produced by the action of MAO) may be the actual GABA-T inhibitor resulting in elevated GABA levels.

Chronic PLZ treatment also produced a significant increase in Ala levels. Like GABA, Ala is metabolized by a transaminase (Kish *et al.*, 1979), and it is believed that PLZ inhibits this enzyme, resulting in the elevated Ala levels. Baker and Martin (1989) have reported that administration of PLZ induced a dose-dependent inhibition of both GABA- and Ala-transaminase in rat whole brain. McKenna *et al.* (1991a) recently confirmed this action of PLZ following its chronic administration. Finding that PLZ can inhibit the transaminase enzymes for both GABA and Ala, it was puzzling to see that the levels of the other measured amino acids Leu, Ileu, Val were not altered, when several of them are known to be metabolized by pyridoxal phosphate-dependent transaminases (Chandler, 1989). Why the transamination of GABA or Ala is more sensitive to inhibition by PLZ is not at present known, but differences in the transaminases themselves or their interaction with PLZ and its metabolites are likely to be important factors.

Given that chronic PLZ treatment significantly increases brain GABA and Ala concentrations, what is the clinical significance of these elevations? For Ala, there is a paucity of information in the literature regarding its role(s) in the central nervous system and possible involvement in psychiatric disorders. Elevated Ala levels have been reported following administration of the convulsant pentamethylenetetrazole (Clarke *et al.*, 1989) and like Gly, Ala can activate N-methyl-D-aspartate (NMDA) receptors (Thomson, 1989). Of possibly greater clinical relevance is the finding that Ala can be metabolized via transamination reactions to yield pyruvate or lactate (Sturman and Applegarth, 1985; Stryer, 1981). Lactate infusion has previously been shown to precipitate panic attacks in susceptible individuals (Shear, 1986). In addition to being an effective antidepressant, PLZ is used in the treatment of panic disorder (Ballenger, 1986; Hollister, 1986). It has been postulated by Wong *et al.* (1990b) that an increase in brain levels of Ala might reflect some decreased lactate formation and that this effect may contribute to the antipanic effects of PLZ.

Elevated GABA levels may also play a role in the antipanic efficacy of PLZ. Along with PLZ, the benzodiazepines alprazolam, clonazepam and diazepam are effective antipanic agents (Chouinard *et al.*, 1982; Noyes *et al.*, 1984; Spier *et al.*, 1986). As previously discussed, benzodiazepines and GABA are thought to be intimately related via reciprocal interactions at the $GABA_A$/benzodiazepine receptor complex, with benzodiazepines facilitating GABAergic activity (Martin, 1987). Breslow *et al.* (1989) have recently suggested that an enhancement of GABA transmission might be a key pharmacological component in antipanic drug efficacy. It is interesting that Breslow *et al.* (1989) found the $GABA_B$ selective ligand, baclofen, to be significantly more effective than placebo in reducing the number of panic attacks and scores on the Hamilton anxiety scale, Zung scale and Katz-R nervousness subscale. These observations indicate that both $GABA_A$ and $GABA_B$ receptors may be involved in the mechanisms of symptom reduction in the treatment of panic disorders.

E. CHRONIC MAO INHIBITOR AND TRICYCLIC ANTIDEPRESSANT TREATMENT: EFFECTS ON MAO ACTIVITY AND TRICYCLIC DRUG LEVELS

Introduction: As part of the experimental design for all studies described in this thesis project, measurements of tricyclic drug levels and MAO activity were taken to confirm the efficacy of the drug administration protocol. Tricyclic levels were measured with a modification of the procedure by Drebit *et al.* (1988), while MAO activity was measured according to a modification of the method of Wurtman and Axelrod (1963) [see Methods section for details].

Results: The effects of the two doses of PLZ and TCP on MAO activity (expressed as % inhibition) are shown in Fig. 21. ANOVA and Newman-Keuls revealed a significant drug effect on both MAO-A [F(3,32)=105.4, $p<0.05$] and MAO-B [F(3,32)=409.5, $p<0.05$], with each drug dose being significantly different from Veh controls. The tricyclics, DMI and IMI, did not significantly influence MAO activity under the present conditions (Table 12).

Brain levels of DMI and IMI are displayed in Table 13 as ng/g brain tissue. For IMI, levels of its metabolite DMI have also been included. Brain levels for both drugs are in agreement with results from previous studies conducted in this laboratory. A representative gas chromatogram from these determinations is shown in Fig. 22.

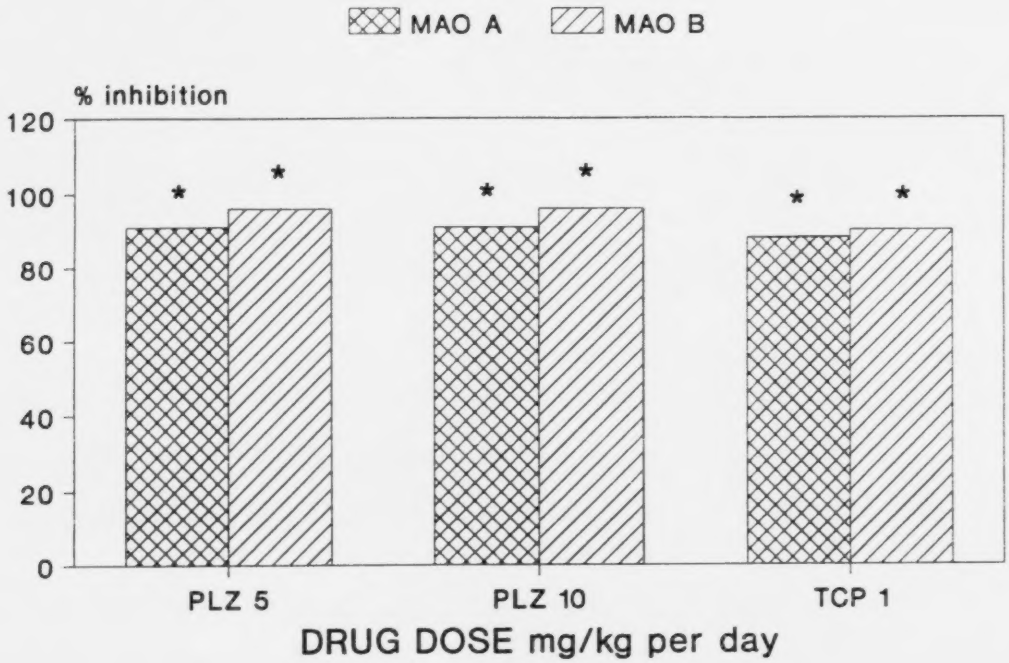


Figure 21: Degree of inhibition of MAO-A and MAO-B in brain following chronic MAO inhibitor administration. * $p < 0.05$ compared to vehicle controls.

% Inhibition

Drug Treatment	Dose mg/kg/d	MAO-A	MAO-B
DMI	5	-1.2 ± 4.8	-0.7 ± 3.8
DMI	10	3.2 ± 3.1	-0.4 ± 1.7
IMI	30	1.6 ± 3.7	-3.0 ± 3.1

Table 12: Degree of inhibition of MAO-A and MAO-B in brain following chronic tricyclic antidepressant treatment. Values are mean ± SEM, N=8, dose mg/kg/d.

Drug Treatment	**Dose mg/kg/d**	**Drug Levels (ng/g)**
DMI	5	DMI 815.4 ± 115
DMI	10	DMI 3485 ± 995
IMI	30	IMI 2683 ± 239
IMI	30	DMI 7912 ± 2992

Table 13: Tricyclic drug levels following their chronic administration. Values are mean ± SEM, N=8, dose mg/kg/d.

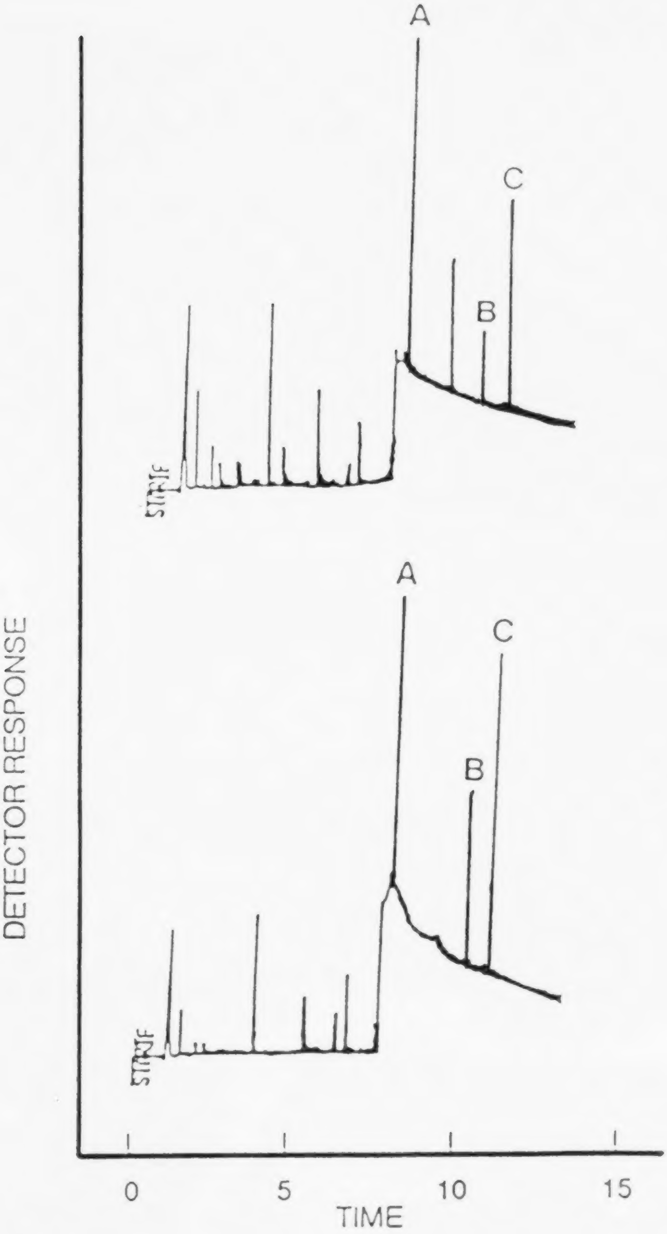


Figure 22: Gas chromatograms from the tricyclic level determinations with homogenized rat brain (top) and authentic standards (bottom). Peaks are: A=IMI; B=N-acetylDMI; C=N-acetylmaprotiline (internal standard).

Discussion: As expected, the chronic (28 d) administration of TCP and PLZ resulted in a significant inhibition of MAO activity. Greater than 85% inhibition of MAO-A and 90% inhibition of MAO-B was observed with both drugs. There was no difference in the degree of inhibition achieved after 5 mg/kg/d of PLZ and that achieved after either TCP or 10 mg/kg/d of PLZ. This result is particularly intriguing when it is compared to the findings from Section B.1 on the behavioural measure of β-adrenoceptor function in which the induction of β-adrenoceptor subsensitivity by PLZ was dose-dependent (with only the higher 10 mg/kg/d dose attenuating the motor suppressant effects of salbutamol). In addition, the dose-dependent effect of PLZ on β-adrenoceptor function also did not correlate with a differential change in receptor number, as both doses of PLZ reduced β-adrenergic binding sites to the same degree (see Section C.1). These results suggest that neither MAO inhibition nor a reduction in the number of binding sites appears to be a sufficient condition for the induction of a functional change in β-adrenoceptors. It appears that, at least for the MAO inhibitor PLZ, effects other than MAO inhibition and decreased binding site number may contribute to the effects of long-term administration.

One possible factor mediating functional changes in β-adrenoceptor induced by PLZ may be 2-phenylethylamine [McManus *et al.,* 1991]. At a dose of 10 mg/kg/d but not at the lower 5 mg/kg/d dose, PLZ was found to induce a significant elevation in liver 2-phenylethylamine levels. This dose-dependent effect of PLZ on 2-phenylethylamine levels is consistent with the results from the functional tests, where only the higher dose of PLZ induced a functional change in β-adrenoceptors. Further support for the possible involvement of 2-phenylethylamine in the present context is provided by the finding that 2-phenylethylamine is a metabolite of PLZ (Baker *et al.,* 1982; Dyck *et al.,* 1985) and appears to have a neuromodulatory role in its interaction with the biogenic amines dopamine, noradrenaline and 5-HT (Jones and Boulton, 1980; Jones, 1984; Paterson *et al.*, 1990).

As MAO inhibitors like TCP and PLZ have a significant influence on a variety of neuronal systems including monoamine uptake and release (Hendley and Snyder, 1968; Baker *et al.*, 1980; Hampson *et al.*, 1986) and enzymes other than MAO (Perry and Hansen, 1973; Robinson *et al.*, 1979; Yu and Boulton, 1991), an interesting future area of research on the actions of MAO inhibitors would be an assessment of the influence of these effects on their clinical efficacy. It is tempting to speculate that these non-MAO-related effects may be vital for the apparent preferential efficacy of MAO inhibitors in the treatment of atypical depression (Klein, 1975; Baldessarini, 1985b). In support of this suggestion, a comparative report on the effects of IMI and PLZ on plasma 2-phenylethylamine levels by McGrath *et al.* (1988) revealed a substantial increase in plasma 2-phenylethylamine with PLZ treatment but no significant change in 2-phenylethylamine levels following chronic IMI treatment.

A lack of correspondence between degree of MAO inhibition and the induction of functional changes in β-adrenergic receptors by PLZ is also interesting in light of the claim that degree of MAO inhibition might be used as a predictor of therapeutic response to this type of drug (Georgotas *et al.*, 1981). Several clinical studies have demonstrated that in patients receiving PLZ treatment, doses of PLZ sufficient to produce at least 80% inhibition of platelet MAO activity are associated with significantly greater antidepressant and antianxiety efficacy; but if the inhibition is less than 60%, a poor antidepressant effect is obtained (Davidson *et al.*, 1978; Robinson *et al.*, 1978a,b; Raft *et al.*, 1981; Nies and Robinson, 1982; Nies, 1983). In these studies, there were also a number of non-responders who had achieved 80% inhibition of MAO. It has been suggested that other factors such as subtype of depression, number of previous episodes and family history may also be required in addition to high degree of MAO inhibition (Georgotas *et al.*, 1981) for optimal response.

An association between reduced platelet MAO activity and clinical response has not, however, been found for a variety of other MAO inhibitors (Murphy *et al.*, 1985). For the MAO-A-selective inhibitor clorgyline, clinical improvement was observed in association with negligible platelet MAO-B inhibition and greater than 85% MAO-A inhibition (Murphy *et al.*, 1979, 1981). Similarly, very low doses of the partially selective MAO-B inhibitors pargyline and deprenyl were shown to inhibit over 95% of platelet MAO activity within hours, while not consistently resulting in antidepressant effects (Murphy *et al.*, 1985). Giller and Lieb (1980) found that administration of TCP resulted in marked platelet MAO inhibition at clinically sub-therapeutic doses.

The results of the measurement of tricyclic antidepressant drug levels in animals treated with IMI and DMI can be seen in Table 13. In the chronic IMI-treated group, a substantial proportion of the administered dose had been metabolized to the desmethylated metabolite DMI. This result confirms the extensive metabolism of IMI *in vivo* (Bickel and Weder, 1968; Rudorfer and Potter, 1985) and the large accumulation of DMI in brain tissue following chronic IMI treatment (Potter *et al.*, 1979). As indicated by the size of the standard errors, there was also a considerable degree of variability in the amounts of metabolism between animals. Because the sole aim for measuring tricyclic drug levels was to confirm the efficacy of the drug protocol and thus no additional IMI or DMI metabolites were assayed, a comprehensive analysis of differences in tricyclic drug metabolism was not carried out.

GENERAL DISCUSSION

The present study was undertaken to identify the effects of chronic antidepressant drug administration on brain GABAergic activity in hopes of further elucidating the potential role of GABA in the mechanism(s) of action of antidepressant treatments. Previous studies on the effects of repeated antidepressant drugs on GABA_B receptors have not yielded a homogeneous picture. As outlined in Section D.5 of the Introduction, chronic antidepressant drug administration has been found to either increase or have no effect on the number and function of GABA_B receptors in rat brain. Results from the present investigation add considerable negative data to the effect of antidepressants on GABA_B receptors. Chronic treatment with four clinically effective antidepressants (PLZ, TCP, IMI and DMI) all failed to alter either the total number of GABA_B binding sites or their functional sensitivity as assessed with an *in vivo* behavioral drug challenge. Under identical experimental conditions, these antidepressants did, however, induce a significant reduction in both the number and function of cortical β-adrenoceptors. In the salbutamol test of β-adrenoceptor function, chronic antidepressant drug groups were found to have lower baseline response rates, as measured by reduced saline day locomotor activity, compared to vehicle controls (see Appendix II). It is unlikely, however, that this baseline effect could account for the attenuated response to salbutamol observed following chronic antidepressant treatment. The same antidepressant drugs were administered in both the β-adrenoceptor and GABA_B receptor behavioural tests and reduced baseline locomotor activity in both tests. However, a differential effect of the antidepressant treatments was found in the two tests: reduced behavioural effects of β-adrenoceptor stimulation, but no change in GABA_B receptor responsiveness. If the baseline effect was the basis for the β-adrenoceptor change, no differential effect in the two behavioural tests would have been seen. The parallel analysis of antidepressant-induced changes in β-adrenoceptors was included to provide an important comparison for the assessment of $GABA_B$ receptor changes. As previously mentioned, decreases in β-adrenoceptor density and function are two of the most commonly observed emergent receptor changes following repeated antidepressant treatment. In the present study, changes in the total number of cortical β-adrenoceptors were assessed using the non-selective β-adrenoceptor antagonist $[^3H]$-DHA. It has been suggested [see Riva and Creese (1989)] that $[^3H]$-DHA may not be the most suitable radioligand for investigating the regulation of β-adrenoceptors by pharmacological treatments. Recent binding experiments conducted in the Neurochemical Research Unit with the novel β-adrenoceptor antagonist $[^3H]$-CGP-12177 have, however, confirmed the reported β-adrenoceptor number changes using the same antidepressant drug treatments and chronic (28 d) drug delivery period (Paetsch *et al.,* 1991a,b).

Recently, it has been suggested that a GABAergic-noradrenergic interaction may be of importance to the role of GABA in antidepressant drug action (Dennis *et al.,* 1985; Dennis and Scatton, 1985; Suzdak and Gianutsos, 1985a,b, 1986; Lloyd *et al.,* 1990). There is a good deal of evidence for a functional coupling between $GABA_A$, $GABA_B$ and β-adrenoceptors in brain. *In vivo* administration of $GABA_A$ receptor agonists has been shown to increase, whereas $GABA_B$ receptor agonists decrease, the release of noradrenaline (Anden and Wachtel, 1977; Suzdak and Gianutsos, 1985b). Repeated treatment with either the $GABA_A$ agonist THIP or the $GABA_B$ agonist baclofen decreases β-adrenergic binding (Suzdak and Gianutsos, 1985a). Baclofen, but not $GABA_A$ receptor agonists, has been shown to potentiate the noradrenaline-stimulated cAMP production in cortical slices (Karbon *et al.,* 1984; Hill *et al.,* 1984; Suzdak and Gianutsos, 1985b). Suzdak and Gianutsos (1985a,b, 1986) have done considerable work on the interaction between $GABA_B$ and β-adrenoceptors at the level of the cAMP generating system, and have demonstrated that this second messenger system and the regulatory $GABA_B$ and β-adrenergic inputs on it are modified by antidepressant treatment. In cortical brain slices, chronic IMI reduced both β-adrenoceptor binding and noradrenaline-stimulated cAMP accumulation. In addition, chronic IMI also increased the ability of baclofen to potentiate the noradrenaline increase in cAMP. Interestingly, IMI treatment did not alter the inability of baclofen alone to affect cAMP levels. It was concluded by these authors that $GABA_B$ receptor stimulation fine-tunes postsynaptic noradrenergic activity in response to drug treatment and that this action may be involved in the therapy of affective disorders (Suzdak and Gianutsos, 1986). Given the high degree of interaction between these two neurotransmitter systems, it is tempting to speculate that in addition to the likely involvement of $GABA_B$ receptor subtypes, another possible factor in the variable antidepressant-induced $GABA_B$ receptor changes that have been observed might be a ratio or balance in the degree of modification (down-regulation of β-adrenoceptors, up-regulation of $GABA_B$ receptors) that is required to bring about a desired effect (e.g. normalization of neuronal activity).

The present study also examined the effects of repeated antidepressant treatment on brain GABA metabolism. It was demonstrated that of the four antidepressants tested, only the MAO inhibitor PLZ altered GABA levels or activity of the GABA metabolic enzyme GABA-T.

CONCLUSIONS

1. *In vivo* behavioral tests of β-adrenoceptor and $GABA_B$ receptor function revealed that chronic administration of antidepressant drugs from both MAO inhibitor and tricyclic drug classes all induced a functional down-regulation of β-adrenoceptors but did not alter the functional sensitivity of $GABA_B$ receptors.

These data extend and confirm previous reports of functional changes in β-adrenoceptors, but not of $GABA_B$ receptors, and do not provide evidence that chronic antidepressant treatments induce a functional increase in the number of $GABA_B$ receptors.

2. Changes in the density and affinity of cortical β-adrenergic and $GABA_B$ binding sites were analyzed with *ex vivo* radioligand binding procedures. All antidepressants tested induced a reduction in the density of β-adrenergic binding sites, but did not significantly alter the density of $GABA_B$ binding sites. No effects on binding site affinity were observed. These results do not replicate the findings of Lloyd *et al.* (1985) and do not support the proposal that an increase in the total population of $GABA_B$ binding sites is a general consequence of chronic antidepressant drug treatment.

3. Chronic administration of PLZ induced a dose-dependent decrease in the functional sensitivity of β-adrenoceptors. This functional down-regulation was not, however, paralleled by a dose-dependent decrease in either binding site number or inhibition of MAO. These data indicate that neither a reduction in cortical binding site number nor MAO inhibition is a sufficient condition for the induction of a functional change in β-adrenoceptors.

4. Radioligand binding analysis of changes in $GABA_A$ binding sites indicated that chronic antidepressant treatment does not modify high affinity $GABA_A$ binding sites.

5. Long-term treatment with PLZ, but not with IMI, DMI or TCP, induced a significant inhibition of GABA-T activity and elevation in GABA levels. These effects of PLZ on GABA metabolism may play a role in its efficacy as an antidepressant/antipanic drug.

POSSIBLE FUTURE RESEARCH

Results from the present experiments suggest a number of avenues that should be investigated with regard to the role of the GABAergic system in the mechanism(s) of action of antidepressant drugs:

1. Recent evidence from pharmacological studies have suggested the existence of distinct subtypes of $GABA_B$ receptors. Radioligand binding studies should be carried out with selective ligands for these subtypes [(-)-baclofen, 3-aminopropylphosphinic acid (3-APA) and saclofen] in various brain regions following chronic antidepressant drug treatment. Data from these studies may help explain the conflicting binding and functional test results found for the action of chronic antidepressants on $GABA_B$ receptors.

2. Effects of repeated treatment with antidepressants on radioligand binding (e.g. $[^3H]$-flunitrazepam) at benzodiazepine binding sites. This study should also include a measurement of the ability of GABA to facilitate benzodiazepine binding so as to allow for an analysis of changes at both benzodiazepine sites (directly) and low affinity $GABA_A$ sites (indirectly) on the $GABA_A$/benzodiazepine receptor-chloride ionophore complex.

3. Comparison of the effects of chronic antidepressants on radioligand binding to $GABA_A$, $GABA_B$ and β-adrenoceptors in brain regions from the same animals. There is a significant amount of data suggestive of a functional coupling between

these two neurotransmitter systems (see Suzdak and Gianutsos, 1985a,b, 1986) and this proposed study would allow for a direct comparison of receptor changes in both receptor systems. This analysis should further our knowledge of the importance of GABAergic-noradrenergic interactions in the mechanisms of action of antidepressant treatments.

A second component of this study could include an analysis of changes in the cAMP-generating system linked to both $GABA_B$ and β-adrenoceptor activation. Levels of cAMP would be measured in brain slices and brain homogenates following: [a] addition of β-adrenoceptor agonist alone (e.g. isoproterenol or noradrenaline); [b] addition of $GABA_B$ receptor agonist alone (e.g. baclofen or GABA); [c] addition of $GABA_B$ and β-adrenoceptor agonists in combination; and [d] basal cAMP levels.

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APPENDIX I

F-values for effects of chronically administered antidepressant drugs on frontal cortex amino acid levels.

GABA	$[F(4,30) = 9.62, p < 0.05]$
Alanine	$[F(4,30) = 3.83, p < 0.05]$
Valine	$[F(4,30) = 0.32, p > 0.05]$
Leucine	$[F(4,30) = 1.20, p > 0.05]$
Isoleucine	$[F(4,30) = 1.67, p > 0.05]$

APPENDIX II

Saline Day Locomotor Activity Counts

Drug Treatment	Dose mg/kg/d	Salbutamol	(±)-Baclofen	Progabide
Vehicle		2130 ± 181 (23)	2493 ± 198 (24)	2224 ± 173 (26)
PLZ	5	2262 ± 324 (8)	2636 ± 337 (8)	1829 ± 199 (8)
	10	1571 ± 273 (8)	2375 ± 352 (8)	1872 ± 218 (8)
TCP	1	1859 ± 267 (8)	2844 ± 379 (7)	1816 ± 197 (10)
IMI	30	1610 ± 237 (8)	2107 ± 185 (8)	1560 ± 184 (10)
DMI	5	2150 ± 465 (8)	2129 ± 257 (8)	1196 ± 216 (6)
	10	1271 ± 144 (8)	1984 ± 253 (6)	1515 ± 162 (8)

Counts are expressed as the total number of beam breaks.

Numbers in parentheses are number of animals per treatment group.